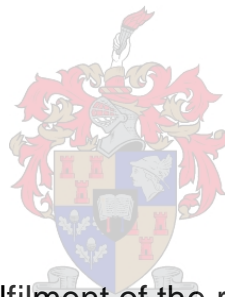


# Phenolic extraction of South African red wines

by

**Elzanne Fourie**



Thesis presented in partial fulfilment of the requirements for the degree of  
**Master of Agricultural Sciences**

at

**Stellenbosch University**

Viticulture and Oenology, Faculty of AgriSciences

*Supervisor:* Prof. Wessel du Toit

*Co-supervisor:* Dr. José Luis Aleixandre-Tudo & Dr. Mihaela Mihnea

December 2019

## Declaration

By submitting this thesis electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the sole author thereof (save to the extent explicitly otherwise stated), that reproduction and publication thereof by Stellenbosch University will not infringe any third party rights and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

Date: December 2019

Copyright © 2019 Stellenbosch University  
All rights reserved

## Summary

Phenolic compounds are well known for their crucial role in red wine quality parameters such as colour, flavour and mouthfeel attributes. Knowledge about phenolic extraction during fermentation and possible wine practices or techniques to possibly modify phenolic content during red wine production is becoming a necessity in the wine industry to improve overall quality. In addition, the industry requires suitable, rapid, accurate and affordable monitoring tools to be able to improve and modify phenolic content during the process. In this study phenolic levels of fermenting samples were quantified through UV-Visible spectroscopy in combination with PLS calibration models. Furthermore, phenolic extraction was evaluated with batch statistical process control as a statistical monitoring tool.

Eight grape batches of the commercial cultivars Cabernet Sauvignon and Shiraz were randomly selected from the Western Cape wine region. Wines were pressed at 1/3<sup>rd</sup>, 2/3<sup>rd</sup>s and near the end of alcoholic fermentation and two punch down regimes were evaluated for each pressing time. Standard punch down (C<sup>b</sup>) consisted of 3 punch downs per day during the maceration stage of skin contact, whereas increased punch down (T<sup>a</sup>) consisted of 12 punch downs applied during different stages of fermentation. Firstly, significant variance was observed between grape batches for the four measured phenolic parameters (anthocyanins, tannins, colour density and total phenolics), with less prominent differences observed for colour density between batches. Furthermore, soft independent modelling of class analogy (SIMCA) showed wines classified according to the grape batch. Moreover, wines produced under different maceration conditions separated according to the vineyard the grapes were sourced from in the batch level model (BLM). All of the analysis indicated significant phenolic variance between grape batches.

In the BLM OPLS-DA model the fermentation samples collected during maceration separated according to the different pressing times for both cultivars, regardless of punch down level. Fermenting samples obtained from pressing time 1 were associated with low phenolic content, whereas wines pressed at 2/3<sup>rd</sup>s and near the end of fermentation showed trends of higher phenolic content. The BLM OPLS-DA between pressing time 2 and 3 revealed clearer trends for Cabernet Sauvignon, whereas fewer phenolic differences were observed for Shiraz samples. Fermenting samples obtained from pressing time 2 showed higher levels of anthocyanins in comparison with wines pressed near the end of fermentation were associated with higher levels of tannin, polymeric phenol and gallic acid. Shiraz fermenting samples pressed at time 3 showed only the latter. Results indicated Cabernet Sauvignon may possibly be a better suited cultivar for longer maceration, since fermentations pressed near the end of fermentation i.e. longer skin maceration, were associated with higher tannin, polymeric phenol and pigment content. In addition, results also reflected phenolic extraction as a diffusion process driven by maceration length.

Furthermore, evaluating the BLM OPLS-DA a poorer separation was observed between standard and increased punch down frequency. Increased punch down frequency showed trends of higher phenolic content, whereas fermenting samples produced with three punch downs a day was strongly associated with high phenolic acid content only. Phenolic differences were observed between Cabernet Sauvignon and Shiraz indicating results may be batch or variety dependent. Additionally, taking a closer look at both punch down levels for each pressing time, similar results were observed in the OPLS-DA models.

Overall, BSPC allowed for the monitoring of phenolic extraction and identification of possible deviations during maceration. However, this study data was only evaluated after process completion. This methodology could be potentially used to monitor phenolic extraction in real time for future red wine fermentations. Additionally, the approach of PLS calibrations proved to be a suitable, rapid, accurate and cost-effective method to measure phenolic levels of fermenting samples.

## Opsomming

Fenoliese komponente is veral bekend vir hulle belangrike rol in rooiwijn kwaliteit parameters soos kleur, geur en mondgevoelskenmerke. Kennis oor fenoliese ekstrasie gedurende fermentasie en moontlike wynmaak praktyke of tegnieke wat moontlik fenoliese ekstrasie kan modifiseer gedurende rooiwijn produksie word al hoe meer 'n noodsaaklikheid in die wyn industrie om algehele rooiwijn kwaliteit te verbeter. Boonop vereis industrie geskikte, vinnige, akkurate en bekostigbare moniteringsinstrumente om kwaliteit te verbeter, sowel as fenoliese inhoud gedurende die proses te modifiseer. Gedurende die studie is fermentasie monsters se fenoliese vlakke gekwantifiseer deur UV-Sigbare spektroskopie in kombinasie met PLS kalibrasie modelle. Fenoliese ekstrasie was geëvalueer met statistiese prosesbeheer as 'n statistiese moniteringsinstrument.

Agt groepe druive van die kommersiële kultivars Cabernet Sauvignon en Shiraz is geselekteer vanuit die Wes-Kaap wynstreek. Gedurende alkoholiese fermentasie was wyne gepers by  $1/3^{\text{de}}$ ,  $2/3^{\text{des}}$  en naby die einde van alkoholiese fermentasie, sowel as twee deurdruk tegnieke/frekwensies was geëvalueer vir elke periode waarby die druive gepers was. Standaard deurdrucke ( $C^b$ ) het bestaan uit drie deurdrucke per dag gedurende die maserasie tydperk waartydens die doppe in kontak was met die sap, terwyl verhoogde deurdrucke ( $T^a$ ) bestaan het uit 12 deurdrucke per dag gedurende die verskillende fases van fermentasie. Eerstens was beduidende verskille waargeneem tussen die verskillende druif groepe vir die vier fenoliese parameters gemeet naamlik antosianiene, tanniene, kleurdigtheid en totale fenole, met minder prominente verskille tussen druif groepe vir kleurdigtheid. Die analise sagte onafhanklike modellering van klas analogie (SIMCA) het aangedui dat wyne geklassifiseer was volgens die druif groepe waarvan die fermenterende monsters oorspronklik van afkomstig was. Boonop het die wyne wat geproduseer was tydens verskillende maserasie kondisies in die bondelvlakmodel (BLM) geskei volgens die wingerd waarvan die druive afkomstig van was. Alle analyses het daarop gedui dat daar beduidende verskille tussen die druive was ten opsigte van fenoliese komposisie.

In die BLM OPLS-DA model het die fermentasie monsters geskei volgens die verskillende maserasie tyetoe die wyne gepers was, onafhanklik van die deurdruk frekwensie. Fermentasie monsters wat tydens perstyd 1 gedruk was, was geassosieer met lae vlakke van fenoliese inhoud, terwyl wyne wat gepers was by  $2/3^{\text{des}}$  en naby aan die einde van alkoholiese fermentasie het tendense getoon van hoë vlakke van fenoliese inhoud. Die BLM OPLS-DA het meer tendense getoon tussen wyne van perstyd 2 en 3 vir Cabernet Sauvignon, terwyl minder tendense waargeneem is vir Shiraz monsters. Fermentasie monsters van perstyd 2 het tendense getoon van hoër vlakke antosianiene in vergelyking met wyne gepers naby aan die einde van alkoholiese fermentasie wat geassosieer met hoër vlakke van tannien, polimeriese fenol en gallinsuur. Shiraz

fermentasie monsters gepers by tyd 3 het slegs tendense getoon van die laasgenoemde. Resultate dui daarop dat Cabernet Sauvignon moontlik 'n meer geskikte kultivar is vir verlengende maserasie kondisies, aangesien fermentasie monsters gepers naby die einde van alkoholiese fermentasie, m.a.w. verlengde dop maserasie, geassosieerd was met hoër tannien, polimeriese fenol en pigment inhoud. Boonop toon resultate dat fenoliese ekstraksie 'n diffusie proses is gedryf deur die maserasie-lengte.

Na evaluering van die BLM OPLS-DA, was 'n swak skeiding tussen standaard en verhoogde deurdruk frekwensie geobserveer. Verhoogde deurdruk frekwensie het tendense getoon van hoër vlakke van fenoliese inhoud, terwyl fermentasie monsters geproduseer met 3 deurdrukke per dag het slegs tendense getoon van hoër fenoliese suur inhoud. Fenoliese verskille was waargeneem tussen Cabernet Sauvignon en Shiraz wat aandui dat resultate dui dat resultate kultivar of groep afhanklik kan wees. Daarbenewens, evaluering van beide deurdruk frekwensie gedurende die verskillende tye van pers was soortgelyke resultate in die OPLS-DA resultate gevind.

Oor die algemeen het BSPC die monitoring van fenoliese ekstraksie en identifikasie van moontlike afwykings gedurende maserasie toegelaat. Die studie data was egter slegs geëvalueer na proses voltooiing. Die metode kan potensieël gebruik word om fenoliese ekstraksie intyds van toekomstige rooiwijn fermentasies te monitor. Daarbenewens, die gebruik van PLS kalibrasies het bewys dat dit 'n geskikte, vinnige, akkurate en koste-effektiewe metode is om fenoliese vlakke van fermentasie monsters te kwantifiseer.

## Biographical sketch

Elzanne Fourie was born on the 9<sup>th</sup> of January 1994 in a warm sunny Bloemfontein where she grew up and matriculated in 2012 from Oranje Girls School with exceptional academic achievements. After matric she followed her passion and love for wine and enrolled for BSc Agric Viticulture and Oenology at the University of Stellenbosch excited to start her journey and becoming an exceptional individual in the wine industry. She completed her degree in 2016 and enrolled for her master's degree after being identified by the CWG organisation as a top student who excelled in her class. She finished her master's degree in 2019 and was appointed as assistant winemaker at Groot Constantia Wine Estate where she will be given the opportunity to apply her knowledge, she gained over the last 6 years.

## Acknowledgements

I would like to take the following opportunity to acknowledge the exceptional individuals who started and finished this fulfilling journey with me, and I just want to express my appreciation and gratefulness towards them:

- My supervisor, **Prof Wessel du Toit**, for all his input, motivation, wisdom and understanding over the duration of my project.
- My co-supervisor **Dr José Luis Aleixandre-Tudo**, for his patience, guidance and understanding as well as all the support and encouragement when the tough got going. Thank you for all your assistance, patience and advice the last few years.
- My co-supervisor **Dr Mihaela Mihnea** for her assistance and guidance with my project.
- **Jeanne Brand and Valeria Panzeri** for their practical assistance and input with sensorial experimental analyses as well as **Prof Martin Kidd** for his help and guidance with statistical analysis.
- My late Father, **Jan Fourie**, for all his guidance, love and encouragement throughout the years. I am so blessed for the opportunities that he gave me and thanks to him, I am the person I am today. Thank you so much dad and I know you are looking down with a big smile.
- My loving husband, **Marco Bosman**, for motivating and encouraging me throughout my journey. Thank you for being my anchor and loving supporting system.
- My family and friends for their support, physically and emotionally, prayer and encouragement throughout my journey since I started my degree in 2013.
- A big and special thank you to **CWG** and **Winetech** for financing my project and all the research along with it.
- Last, but not least, thank you to our **High and Almighty God**, for protecting and guiding me throughout my life and giving me the knowledge and intelligence to pursue my passion in life and especially the strength over the last two years.



## Preface

This thesis is presented as a compilation of four chapters. Each chapter is introduced separately and is written according to the style of Harvard citation.

**Chapter 1**      **General introduction and project aims**

**Chapter 2**      **Literature review**  
Phenolic compounds and their monitoring during fermentation

**Chapter 3**      **Research results**  
The effect of different maceration conditions on the extraction of phenolic compounds - A batch statistical process control approach.

**Chapter 4**      **General discussion and conclusions**

# Table of Contents

<b>Chapter 1. General introduction and project aims</b>	<b>1</b>
1.1 Introduction	2
1.2 Aims and objectives	3
1.3 References	4
<b>Chapter 2. Literature review</b>	<b>6</b>
2.1 Introduction	7
2.1.1 Flavonoids	7
2.1.2 Non-flavonoids	11
2.2 Factors influencing phenolic concentration in grapes	12
2.2.1 Grape variety	12
2.2.2 Grape ripeness	13
2.2.3 Viticulture practices	14
2.3 Variables and practices influencing phenolic extraction during alcoholic fermentation	15
2.3.1 Maceration length and increasing alcohol content	15
2.3.2 Cap management	18
2.3.3 Fermentation temperature	19
2.3.4 Macerating enzymes	20
2.3.5 Grape composition	21
2.4 Quantification of phenolic compounds during alcoholic fermentation	22
2.4.1 Reference methods for phenolic analysis	23
2.4.1.1 Quantification of wine colour	23
2.4.1.2 Quantification of anthocyanins	23
2.4.1.3 Quantification of total phenols	24
2.4.1.4 Quantification of tannins	24
2.4.1.5 Quantification of individual phenolics	24
2.5 Statistical analysis used for wine-related data	25
2.5.1 Batch statistical process control (BSPC)	25
2.6 Conclusion	26
2.7 References	27
<b>Chapter 3. Research results</b>	<b>35</b>
3.1 Introduction	36
3.2 Materials and Methods	37
3.2.1 Reagents	37
3.2.2 Experimental design	38
3.3.3 Winemaking	39
3.3.4 Analysis	39
3.3.5 Statistical analysis	41
3.3 Results	43
3.3.1 Variability of grape and wine phenolics	43
3.3.2 The effect of pressing time (absence/presence of skins) on phenolic extraction	48

during alcoholic fermentation	
3.3.3 The effect of punch down frequency applied during maceration	52
3.3.4 Phenolic levels of the final wines	58
3.4 Discussion	59
3.4.1 Vineyard compositional variation in grape and wine samples	59
3.4.2 The influence of skin contact time on phenolic content	59
3.4.3 The effect of punch down frequency applied during maceration	62
3.5 Conclusion	63
3.6 References	63
3.6 Supplementary information for Chapter 3	67
<b>Chapter 4. General conclusions and recommendations</b>	<b>78</b>
4.1 General conclusions	79
4.2 Future recommendations	80

# Chapter 1

---

## Introduction and project aims

## 1.1 INTRODUCTION

Red wine phenolics can be defined as a complex and diverse group of grape-derived substances classified in sub-phenolic groups based on their chemical composition and structure (Casassa and Harbertson, 2014; Fulcrand *et al.*, 2006; Teixeira *et al.*, 2013). Although phenolic compounds represent less than 5% of the total composition in red wine, with ethanol and water contributing 95% of the total content, phenolic compounds are of great interest, due to their impact on red wine quality and possible health benefits (Aleixandre-Tudo *et al.*, 2017). Moreover, anthocyanins, monomeric and polymeric flavanols have been identified as the most influential phenolic substances in red wine, influencing both colour and mouthfeel properties (Cheynier *et al.*, 2006). In addition, polymeric pigments are formed as a result of anthocyanins and polymeric flavanols reactions, contributing towards long term colour stabilization. Polymeric flavanols, also known as tannins, are well known for their role in mouthfeel attributes such as bitterness and astringency, as well as their influence on colour stability and aging potential of wines (Lorenzo *et al.*, 2005; Monagas *et al.*, 2005). On the other hand, colour has been identified as the most easily recognized quality marker in red wine, influenced by anthocyanins (Sacchi *et al.*, 2005). Anthocyanins have been classified as the red compounds responsible for colour of both grapes and wine, with polymeric pigments also contributing towards red wine colour (Monagas *et al.*, 2005). Due to the nature of these phenolic substances, several studies have investigated numerous winemaking techniques and practices to possibly modify and enhance phenolic content and improving overall red wine quality (Sacchi *et al.*, 2005; Smith *et al.*, 2015).

Since the desired phenolic compounds are located in the grape skins and seeds, maceration is a common technique used to extract phenolic compounds from the grape pomace into the fermenting must during red wine production (Lerno *et al.*, 2015). The term macerate is defined as a softening process by soaking in liquid, thus grape skins are left on the juice/wine before, during or after alcoholic fermentation to extract desired phenolic compounds (Nel *et al.*, 2014). A number of variables such as temperature, sufficient contact between the solids and liquid, skin contact time and grape composition have been reported as important factors influencing extraction during maceration (Koyama *et al.*, 2007; Smith *et al.*, 2015). However, management of skin contact time has been reported as the most crucial factor influencing phenolic content (Kelebek *et al.*, 2006; Romero-Cascales *et al.*, 2005; Zimman *et al.*, 2002). It is well known that phenolics have different extraction kinetics, particularly skin and seed-derived phenolic compounds, primarily influenced by maceration length and conditions (Canals *et al.*, 2005). Numerous authors have investigated anthocyanin and tannin extraction during alcoholic fermentation, although the influence of skins present during different stages of fermentation has not yet been thoroughly investigated (Romero-Cascales *et al.*, 2005; Hernández-Jiménez *et al.*, 2012). On the other hand, it is well known that sufficient contact between the skins, seeds and juice influences phenolic content, however little research has been done regarding cap management (Smith *et al.*, 2015). The degree to which the cap is in contact with the must may particularly influence phenolic extraction and needs to be further investigated (Cerpa-Calderón and Kennedy, 2008).

On the other hand, a simplistic, robust and cost-effective method is of great importance to monitor phenolic extraction during maceration as well as to quantify phenolic content of fermenting samples (Aleixandre-Tudo *et al.*, 2018). Several spectrophotometric based methods for the analysis of phenolic content have been recently evaluated in a bibliometric study for both grape and wine (Aleixandre-Tudo *et al.*, 2017). Spectrophotometric analysis is therefore a common analytical method used in wine research to estimate phenolic content in both grape and wine (Harbertson and Spayd, 2006; Aleixandre-Tudo *et al.*, 2018). Due to the UV-Visible light absorbing nature of these phenolic substances, the use of an UV-Visible-based spectrophotometric method thus appears as a suitable approach to quantify phenolic content. Additionally, UV-Visible spectroscopy seems to be a more cost-effective and available technique in comparison with infrared analysis. Moreover, quantification of individual phenolic compounds has been reported as well, following high performance liquid chromatography (HPLC) (Peng *et al.*, 2002). However, HPLC analysis, that can be used to quantify individual phenolics, is a time consuming and a complex approach, which requires skilled personnel (Aleixandre-Tudo *et al.*, 2018). Limited research has been publicised on the continuous monitoring of phenolic extraction during alcoholic fermentation consisting of a large number of samples, which were probably due to analytic and data processing limitations. Alternatively, the combination of spectroscopy with chemometrics has been of great interest, since this method offers the potential to simplify and reduce the analytical time as well as provide a more cost-effective approach to simultaneously measure several phenolic compounds from a single spectral measurement (Cozzolino, 2015). The effectiveness of UV-Visible spectroscopy to quantify phenolic levels of fermenting samples as well as of finished wines has been shown in numerous studies (Damberg *et al.*, 2012; Aleixandre-Tudo *et al.*, 2015). In spectroscopy applications the quantification is based on spectral data collection once an accurate calibration has been obtained. The use of UV-Visible spectral data has been proposed as a suitable alternative for phenolic analysis during the fermentation process (Aleixandre-Tudo *et al.*, 2018). In combination with an appropriate multivariate statistical data analysis approach, valuable information on process progression as well as overall process performance can be obtained (Aleixandre-Tudo *et al.*, 2019).

## 1.2 AIMS AND OBJECTIVES

The overall aim of this research was to investigate the extraction of phenolic compounds during the fermentation process of wines made under different maceration conditions and winemaking practices. To achieve this main aim, the following objectives had to be met:

- i. Punch down was used as an efficient technique to possibly enhance phenolic extraction during the maceration step and possibly ensure optimum contact between the seeds, skins and fermenting juice. The influence of punch down frequency was therefore evaluated by comparing a standard punch down procedure with increased punch down frequency.
- ii. As highlighted before, authors have proposed that the control of the maceration length to be the most critical factor influencing phenolic extraction and content. Since, management of the maceration length is supposed to be the most effective variable to modify phenolic content, the effect of the

presence/absence of skins on phenolic content was investigated by pressing at different stages during alcoholic fermentation.

- iii. To account for potential varying effect of cap management and maceration length practices in different cultivars and grape batches, phenolic variability was assessed by including eight different batches of Shiraz and Cabernet Sauvignon.
- iv. In addition, the suitability of batch statistical process control to monitor phenolic content in red wine fermentations making use of PLS spectroscopy calibrations was evaluated. The approach was compared with existing literature, focussing on studies reporting on phenolic extraction kinetics.

## 1.3 REFERENCES

- Aleixandre-Tudo, J. L. and du Toit, W. J. (2019). Understanding cold maceration in red winemaking: A batch processing and multi block data analysis approach. *Food Sci. Tech.*, 111, 147-157.
- Aleixandre-Tudo, J. L., Buica, A., Nieuwoudt, H. and du Toit, W.J. (2017). Spectrophotometric analysis of phenolic compounds in grapes and wines. *J. Agric. Food Chem.*, 65, 4009-4026.
- Aleixandre-Tudo, J. L., Nieuwoudt, H., Olivieri, A. and du Toit, W.J. (2018). Phenolic profiling of grapes, fermenting samples and wines using UV- Visible spectroscopy with chemometrics. *Food Control*, 85, 11-22.
- Aleixandre-Tudo, J. L., Nieuwoudt, H. and du Toit, W. J. (2015). Robust ultraviolet-visible (UV-vis) partial least-squares (PLS) models for tannin quantification in red wine. *J. Agric. Food Chem.*, 63, 1088-1098.
- Canals, R., Llaudy, M. C., Valls, J., Canals, J. M. and Zamora, F. (2005). Influence of ethanol concentration on the extraction of color and phenolic compounds from the skin and seeds of Tempranillo grapes at different stages of ripening. *J. Agric. Food Chem.*, 53, 4019-4025.
- Lorenzo, C, Pardo, F, Zalacain, A, Alonso, G. L. and Salinas, M. R. (2005). Effect of red grapes co-winemaking in polyphenols and color of wines. *J. Am. Food Chem.*, 53(19), 7609-7616.
- Casassa, L. F. and Harbertson, J. F. (2014). Extraction, evolution, and sensory impact of phenolic compounds during red wine maceration. *Annu. Rev. Food Sci. Technol.*, 5, 83-109.
- Cerpa-Calderón, F. K. and Kennedy, J. A. (2008). Berry integrity and extraction of skin and seed proanthocyanidins during red wine fermentation. *J. Agric. Food Chem.*, 56(19), 9006-9014.
- Cozzolino, D. (2015). The role of visible and infrared spectroscopy combined with chemometrics to measure phenolic compounds in grape and wine samples. *Molecules*, 20, 726-737.
- Cheynier, V., Salas, E., Souquet, J. M., Sarni-Manchado, P. Maury, C. and Fulcrand, H. (2006). Structure and properties of wine pigments and tannins. *Am. J. Enol. Vitic*, 57(3), 298–305.
- Damberg, R. G., Mercurio, M. D., Kassara, S., Cozzolino, D. and Smith, P. A. (2012). Rapid measurement of methyl cellulose precipitable tannins using ultraviolet spectroscopy with chemometrics: Application to red wine and inter-laboratory calibration transfer. *Appl. Spectrosc.*, 66(6), 656-664.
- Fulcrand, H., Dueñas, M., Salas, E. and Cheynier, V. (2006). Phenolic reactions during winemaking and aging. *Am. J. Enol. Vitic.*, 57(3), 289-297.
- Harbertson, J. F. and Spayd, S. (2006). Measuring phenolics in the winery. *Am. J. Enol. Vitic.*, 57(3), 280-288.
- Hernández-Jiménez, A., Kennedy, J. A., Bautista-Ortín, A. B. and Gómez-Plaza, E. (2012). Effect of ethanol on grape seed proanthocyanidin extraction. *Am. J. Enol. Vitic.*, 63, 57-61.
- Kelebek, H., Canbas, A., Selli, S., Saucier, C., Jourdes, M. and Glories, Y. (2006). Influence of different maceration times on the anthocyanin composition of wines made from *Vitis vinifera* L. cvc. Boğazkere and Öküzgözü. *J. Food Eng.*, 77, 1012-1017.
- Koyama, K., Goto-yamamoto, N. and Hashizume, K. (2007). Influence of maceration temperature in red wine vinification on extraction of phenolics from berry skins and seeds of grape (*Vitis vinifera*). *Biosci. Biotechnol. Biochem.*, 71(4), 958-965.
- Lerno, L., Reichwage, M., Ponangi, R., Hearne, L., Block, D. E. and Oberholster, A. (2015). Effects of cap and overall fermentation temperature on phenolic extraction in Cabernet Sauvignon

- fermentations. Am. J. Enol. Vitic., 66(4), 444-453.
- Monagas, M., Bartolomé, B. and Gómez-Cordovés, C. (2005). Updated knowledge about the presence of phenolic compounds in wine. Crit. Rev. Food Sci. Nutri., 45, 85-118.
- Monagas, M., Gómez, G. and Bartolomé, B. (2005). Evolution of polyphenols in red wines from *Vitis vinifera* L. during aging in the bottle. I. Anthocyanins and pyranoanthocyanins. Eur. Food. Res. Technol., 220, 607-614.
- Nel, A. P., van Rensburg, P. and Lambrechts, M. G. (2014). The influence of different winemaking techniques on the extraction of grape tannins and anthocyanins. S. Afri. J. Enol. Vitic., 35(2), 304-320.
- Peng, Z., Iland, P. G., Oberholster, A., Sefton and Waters, E. J. (2002). Analysis of pigmented polymers in red wine by reverse phase HPLC. Aus. J. Grape Wine Res., 8, 70-75.
- Romero-Cascales, I., Fernández-Fernández, J. I., López-Roca, J. M. and Gómez-Plaza, E. (2005). The maceration process during winemaking extraction of anthocyanins from grape skins into wine. Eur. Food. Res. Technol., 221, 163-167.
- Sacchi, K. L., Bisson, L. F. and Adams, D. O. (2005). A review of the effect of winemaking techniques on phenolic extraction in red wines. Am. J. Enol. Vitic., 56(3), 197-206.
- Smith, P. A., Mcrae, J. M. and Bindon, K. A. (2015). Impact of winemaking practices on the concentration and composition of tannins in red wine. Aus. J. Grape Wine Res., 21, 601-614.
- Teixera, A., Eiras-Dias, J., Castellarin, S. D. and Gerós, H. (2013). Berry phenolics of grapevine under challenging environments. Int. J. Mol. Sci., 14, 18711-18739.
- Zimman, A., Joslin, W., Lyon, M., Meier, J. and Waterhouse, A. (2002). Maceration variables affecting phenolic composition in commercial-scale Cabernet Sauvignon winemaking trials. Am. J. Enol. Vitic., 53(2), 93-98.



# Chapter 2

---

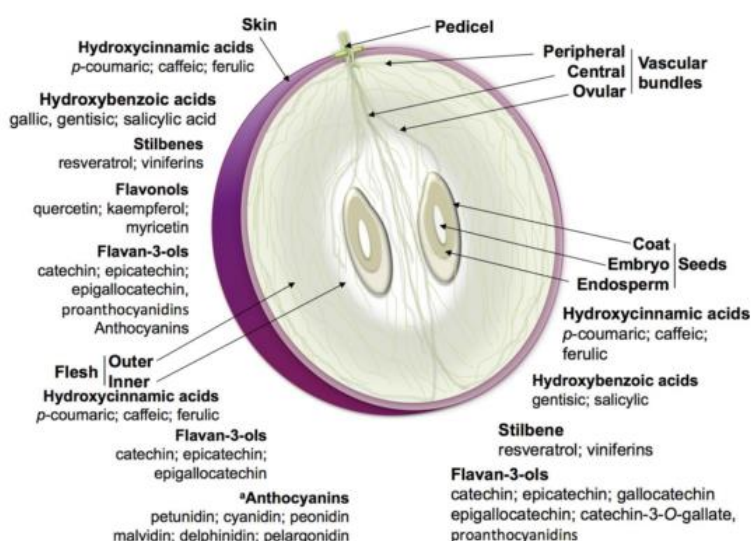
## Literature review

### Phenolic compounds and their monitoring during fermentation

## 2.1 INTRODUCTION

Phenolic compounds contribute to the sensory characteristics, associated with quality parameters, particularly the colour properties, astringency, bitterness and structure of the wine, as well as the ageing potential (Mazza *et al.*, 1999; Sacchi *et al.*, 2005; Koyama *et al.*, 2007; Segade *et al.*, 2008; Monagas *et al.*, 2017). However, phenolic compounds do not only influence the quality of red wine but have also been investigated for their possible influence on human health (De Beer *et al.*, 2006; Petrovic *et al.*, 2012; Monagas *et al.*, 2017).

Phenolics are plant-derived compounds and have been identified in various foods and beverages at different concentrations (Burin *et al.*, 2010; Cerpa-Calderón and Kennedy, 2008). Grape phenolics are structurally and chemically a diverse group of compounds characterized by an aromatic phenol ring containing one or more hydroxyl groups (-OH) (Cheynier *et al.*, 2006; Mattivi *et al.*, 2009). In grape berries, phenolic compounds are mainly located in the skins, seeds and pulp with varying phenolic families found in different tissues (Lerno *et al.*, 2015) (**Figure 2.1**). In addition, phenolic compounds are highly reactive substances and can be classified based on their chemical properties and structure in two main groups known as flavonoid (C6-C3-C6) and non-flavonoid (C6 backbone) compounds (Aleixandre-Tudo *et al.*, 2017; Cheynier *et al.*, 2006).

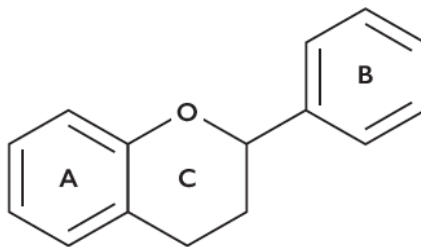


**Figure 2.1:** A schematic structure of flavonoid and non-flavonoid distribution and accumulation in the grape berry.

### 2.1.1 FLAVONOIDS

Flavonoids have a specific three-ring structure with two polyhydroxylated aromatic A and B-rings joined with a heterocyclic C-ring (Fulcrand *et al.*, 2006; Teixeira *et al.*, 2013) (**Figure 2.2**). The flavonoid sub-classes are determined based on the oxidation state of the C-ring (Waterhouse, 2002). The most important flavonoids present in grapes are monomeric and polymeric flavan-3-ols, anthocyanins and flavonols (Teixeira *et al.*, 2013). Other flavonoids present in grapes at very low concentrations are the flavones and flavanonols (Monagas *et*

*et al.*, 2005). Anthocyanins have an unsaturated C-ring in their positively charged flavylum form (red coloured form) compared to flavanols (tannins) which have a saturated C-ring in monomeric and polymeric forms (Fulcrand *et al.*, 2006). Grape flavonoids are located and extracted mainly from the skins and the seeds during maceration (Hanlin *et al.*, 2009; Monagas *et al.*, 2017).



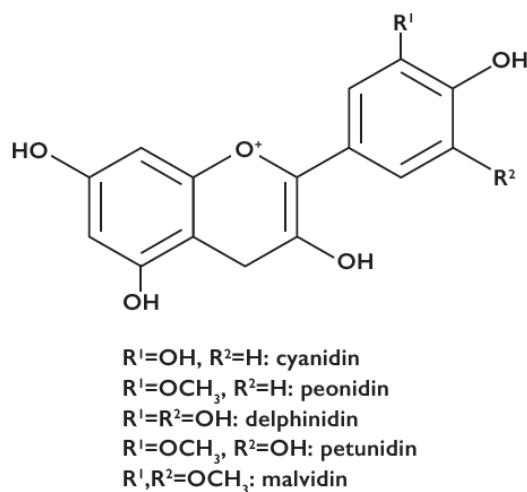
**Figure 2.2:** Characteristic flavonoid ring structure.

### Anthocyanins

Anthocyanins are red pigments located in the vacuoles of grape skin cells responsible for the colour of red grapes and wine (Sacchi *et al.*, 2005; Teixeira *et al.*, 2013). In teinturier varieties anthocyanins are located also in the pulp (Harbertson *et al.*, 2009). Anthocyanins accumulate in the cytosol of the epidermal cells from *veraison*, 8-10 weeks after blooming, until grape maturity, however a decrease may occur in overripe grapes (Braidot *et al.*, 2008; Cerpa-Calderón and Kennedy, 2008).

Anthocyanins are glycosides and acylglucosides of anthocyanidins of which five basic anthocyanidins have been identified in grape skins and in wine: malvidin, cyanidin, delphinidin, peonidin and petunidin (Teixeira *et al.*, 2013) (**Figure 2.3**). Anthocyanins have also been found esterified with phenolic acids such as *p*-coumaric, caffeic and acetic. The form of anthocyanin is dependent on the pH of the wine medium (Monagas *et al.*, 2005). In a study reported by Glories (1984) four different structures were identified at wine pH of 3.5: 12.2% of the anthocyanin structures were in their red flavylum form, 15% in blue quinoidal base, 27.6% as colourless chalcones and 45.2% in colourless carbinol pseudobase. Anthocyanins present in their flavylum form (red colour) are favoured by acidic conditions (low pH). As the pH increases anthocyanins will be present in their quinodal (blue) and colourless chalcone and carbinol forms.

Due to the reactive nature of these compounds polymeric pigments are formed as a result of the anthocyanin reaction with condensed tannins. This reaction starts right after the grapes are crushed and will increase over time, contributing to the colour of wine (Bindon *et al.*, 2014; Herderich and Smith, 2005). Pigmented polymers are important for colour stabilization, because the newly formed pigments are more resistant to pH variation and SO<sub>2</sub> decolouration and can contribute as much as 90% of the colour of aged wines (Herderich and Smith, 2005; Ichikawa *et al.*, 2012; Sacchi *et al.*, 2005).



**Figure 2.3:** Anthocyanidin structures identified in the grape berry.

### Flavonols

Flavonols are yellow pigments located specifically in the berry skin and in some layers of the seed coat. Flavonols in the berry skin range from 0.018-0176 mg/g per berry dependent of grape variety. Flavonols accumulate during the early stages of fruit development towards *veraison* (Adams, 2006; Monagas *et al.*, 2005; Teixeira *et al.*, 2013). These compounds act as UV-protectors against the sun, therefore their concentrations and accumulation is influenced by light exposure (Adams, 2006; Braidot *et al.*, 2008). Flavonols are also involved in copigmentation reactions with anthocyanins (Lerno *et al.*, 2015) due to their ability to act as copigments. Flavonols exist as 3-glycosides of four aglycones: quercetin, myricetin, kaempferol and isorhamnetin in both grapes and wine of *Vitis vinifera* (Casassa and Harbertson, 2014; Monagas *et al.*, 2017).

### Flavan-3-ols

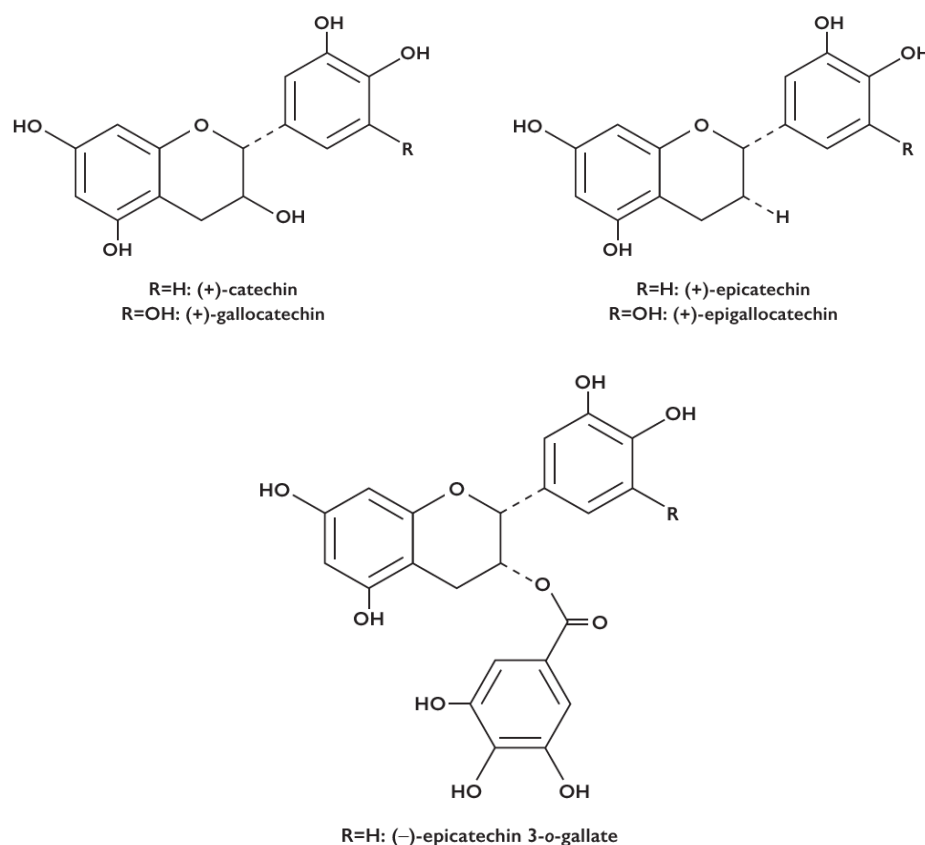
Flavan-3-ols are located in the skins and seeds of grape berries and are present in monomeric and polymeric forms in both grapes and wines. Flavanols accumulate during the first phase of berry growth, slightly declining after *veraison* towards berry ripening (Cerpá-Calderón and Kennedy, 2008; Herderich and Smith, 2005; Ichikawa *et al.*, 2012). During ripening the seeds will start to brown, decreasing seed tannin content available for extraction. This mechanism known as seed browning is due to oxidation of tannins, driven by ripening. The remaining seed tannin available for extraction is influenced by grape variety, cultural practices and winemaking conditions during maceration (Adams, 2006). However seed tannin levels decreases with the progression of ripening, while skin tannin levels can increase (Bindon *et al.*, 2013).

Condensed tannins also known as proanthocyanidins consist of flavan-3-ols polymers of which the subunits consist of (+)catechin, (-)epicatechin, (+)gallocatechin, (-)epigallocatechin and catechin-3-O-gallate in the grape berry (Hanlin *et al.*, 2009; Smith *et al.*, 2015)(**Figure 2.4**). Catechin is the most abundant flavanol monomer present in wine and the second most abundant unit present in condensed tannins compared to its isomer epicatechin which is the most abundant unit present in proanthocyanidins (Adams, 2006; Waterhouse, 2002). Both flavan-3-ols and proanthocyanidins can contribute towards the perception of bitterness and astringency (Monagas *et al.*, 2005). Differences in astringency

perception have been observed between the different flavan-3-ols. Although epicatechin has been perceived and described as more bitter compared to catechin, catechin has been associated with lingering bitterness (Casassa and Harbertson, 2014).

Polymeric proanthocyanidins contribute towards the sensation of astringency experienced upon consumption. One of the proposed mechanisms to explain the astringency sensation experienced is as a result of the protein-tannin complexes formed due to the interaction occurring between proanthocyanidins and the salivary proline-rich proteins (PRPs) located in the oral cavity (Vidal *et al.*, 2004). The aggregation and precipitation of these protein-tannin complexes enhances friction in the oral cavity causing dryness and an astringency sensation, due to a loss of lubrication in the saliva (Ma *et al.*, 2014; Mcrae and Kennedy, 2011). The presence of other wine components such as acids in wine has been reported influencing the perception of astringency experienced with consumption as well (Kallithraka *et al.*, 2007, Noble 1999).

Skin and seed proanthocyanidins are structurally and chemically different (Herderich and Smith, 2005; Adams, 2006; Cerpa-Calderón and Kennedy, 2008). Structurally skin tannins have a much larger mDP (mean degree of polymerization) ranging from 3 to 83 flavanol subunits composed of both prodelphinidins and procyanidins compared to seed tannin (procyanidins), containing only the latter with a much smaller mDP between 2 to 16 subunits (Adams, 2006; Smith *et al.*, 2015). Chemically skin tannin polymers have a higher presence of epigallocatechin compared to seed tannins with a higher presence of catechin, epicatechin and a higher proportion of epicatechin gallate (Adams, 2006; Smith *et al.*, 2015). Both skin and seed proanthocyanidins are sensorially perceived differently, because of their structural and chemical differences. Skin tannins are generally described as having a softer and smoother mouthfeel, causing less dryness and bitterness compared to seed tannin (Hernández-Jiménez *et al.*, 2012).



**Figure 2.4:** Schematic structures of monomeric flavanols, subunits of condensed tannins.

### 2.1.2 NON-FLAVONOIDS

Non-flavonoids consist of a more basic structure (simple C6 backbone) compared to flavonoids and are only present at low levels in grapes and wine (Teixera *et al.*, 2013). The main non-flavonoid compounds are hydroxycinnamic acids, benzoic acids and stilbenes (Adams, 2006).

#### Hydroxycinnamic acids

Hydroxycinnamates are located in the berry skin and pulp and are present as tartaric esters in red wine, free-run juice as well as in white wine. Hydroxycinnamates accumulate in the berry skin, but predominantly in the berry flesh before *veraison* and decrease during the ripening phase as the berry size increases (Teixera *et al.*, 2013). Although hydroxycinnamic acids are more commonly found in white wine, their presence has been reported in red wine as well. The three common hydroxycinnamic acids *p*-coumaric, ferulic and caffeic acid are present in the berry and are *transformed* to coumaric, ferulic and caffeic acid (Monagas *et al.*, 2005). Caffeic acid is the most abundant hydroxycinnamate present in grape and the average level present is about 170 mg/kg (Waterhouse, 2002). Lower levels of ferulic and *p*-coumaric acids are present in the grape berry at respectively 5 mg/kg and 20 mg/kg (Waterhouse, 2002). In finished wines hydroxycinnamates are present in red wines at about 60 mg/L (Waterhouse, 2002).

#### Benzoic acids

The most commonly found hydroxybenzoic acids present in the grape berry are gallic acid, salicylic acid, gentisic acid and *p*-hydroxybenzoic acid which are present as minor compounds in grapes and wine (Monagas *et al.*, 2005). The most known benzoic acid is gallic acid that is present in its free form in the grape berry (Teixera *et al.*, 2013). Gallic acid is a stable compound in aged red wines with levels around 70 mg/L being reported (Waterhouse, 2002).

#### Hydrolysable tannins

Tannins can be categorised in two classes based on their structure and are known as condensed tannin (grape-derived) and hydrolysable tannin (oak-derived) (Herderich and Smith, 2005). Hydrolysable tannin refers to tannins that are extracted from oak barrels or can be added as oenological tannins during vinification (Herderich and Smith, 2005). Hydrolysable tannins are divided into two classes, known as gallotannins and ellagitannins (Waterhouse, 2002). These oak-derived tannins are composed of gallic acid or ellagic acid (Waterhouse, 2002). The concentration of hydrolysable tannins present in red wine is dependent on time spent in the barrel as well as the age and origin of the barrel itself (Smith *et al.*, 2015).

## Stilbenes

Stilbenes are minor non-flavonoid compounds present in the skin of the berry (Monagas *et al.*, 2005). Stilbenes accumulate in the berry skin and are predominant at the ripening phase (Teixera *et al.*, 2013). The concentration of stilbenes vary depending on grape variety and increases in response to fungal attacks (Adams, 2006). Resveratrol also found as resveratrol-glucoside in red wine is a particular stilbene that has been investigated for its possible health benefits towards reducing heart diseases (Petrovic *et al.*, 2012). Resveratrol is more commonly found in red wine at levels of around 7 mg/L (Waterhouse, 2002).

## **2.2 FACTORS INFLUENCING PHENOLIC CONCENTRATION IN GRAPES**

As it has been shown in a large number of studies, the grape phenolic content and composition is greatly influenced by grape variety, environmental factors and viticulture practices applied during the growing season (Bautista-Ortín *et al.*, 2016; Cacho *et al.*, 2000; Flamini *et al.*, 2013; Garrido and Borges, 2010; Ichikawa *et al.*, 2012; Lingua *et al.*, 2016; Segade *et al.*, 2008; Teixeira *et al.*, 2013; Zimman *et al.*, 2002).

### **2.2.1 GRAPE VARIETY**

In an early study, Glories (1999) reported different anthocyanins present in the berry skin, dependent on the variety, with malvidin-3-glucoside being the most predominant anthocyanin found in all grape varieties tested. The anthocyanin fingerprint is primarily determined by the genetic make-up of the variety, known as chemical markers, each with a unique pattern (Romero-Cascales *et al.*, 2005). Several studies have shown that the grape variety influences the concentration, composition and accumulation of anthocyanins in the grape skin (González-Neves *et al.*, 2015; Mazza *et al.*, 1999). This is also in agreement with the study of García-Beneytez *et al.* (2002) who investigated the anthocyanin pattern in grapes and wines of 15 different red cultivars and concluded that each cultivar had a unique anthocyanin profile and pattern. As previously indicated, among them, malvidin-3-glucoside was reported as the most abundant anthocyanin present in wine as well as in most of the skins of fresh grapes, ranging from 32% to 58%. The concentration and composition of other anthocyanin glucosides present in both the skins and wine showed also a large variation that was again highly dependent on the variety. In addition to this, the anthocyanin profile from grape to wine differed due to different anthocyanin extraction kinetics as well as degradation of some of these compounds during the winemaking process.

On the other hand, various studies have reported that certain cultivars are richer in phenolic content compared to others (du Toit and Visagie, 2012; Gambuti *et al.*, 2009). However, tannin profiling of different cultivars is not a common practice. Flavanols are found as monomers, oligomers and polymers of which the polymeric fraction in grapes represents 75-81% in seeds and 94-98% in skins compared to wine where only 77-84% corresponded to polymers (Monagas *et al.*, 2003). Mattivi *et al.* (2009) reported skin-derived grape extracts being high in monomeric catechin, epicatechin, galocatechin and epigallocatechin



compared to seed-derived grape extracts that were rich in monomeric catechin, epicatechin and epicatechin gallate. Skin-derived extracts therefore contained prodelphinidin and procyanidin oligomers while seed-derived extracts only the latter. Catechin was reported as the most abundant flavanol present in the seeds of Pinot noir with epicatechin as the most abundant flavanol present in the seeds of both Merlot and Syrah. Although varieties may have the same abundant flavanol present in the skins or seeds, compositional differences also occur. Based on the information reported, it was thus proven that composition and concentration of tannin present in the skins and seeds is strongly variety dependent. Similar results were also observed in another study investigating flavan-3-ol composition of skins, seeds and wine of three different red varieties (Monagas *et al.*, 2003). The study demonstrated that the concentration and composition of monomeric and polymeric proanthocyanidins are strongly influenced by the variety.

### 2.2.2 GRAPE RIPENESS

The sugar level of the grapes at harvest influences the extraction kinetics of anthocyanins and tannins, the subsequent formation of polymeric pigments as well as the sensory perception of a red wine (Harbertson *et al.*, 2009). Harvesting grapes at a riper degree can result in increased extraction of anthocyanins, tannins and overall total phenolic content (Canals *et al.* 2005), which is in agreement with other studies reporting on the effect of grape maturity on the phenolic content of red wines (Casassa *et al.*, 2013; González-Neves *et al.* 2012; Harbertson *et al.*, 2009; Kennedy *et al.*, 2002).

Although extraction of seed proanthocyanidins is favoured in unripe berries and become less extractable during ripening, harvesting at a higher sugar level can result in higher alcohol formation with the potential increase of seed tannin extraction (Casassa *et al.*, 2013). Moreover, high levels of seed tannin can be associated with negative descriptors like bitterness and astringency (Casassa *et al.*, 2013). Some studies have shown that late harvest is usually associated with increased tannin concentration in wine (Bindon *et al.*, 2014; Bindon *et al.*, 2013; Cadot *et al.*, 2012). This is in agreement with the study of Nel *et al.* (2014) who observed higher tannin extraction in grapes harvested above 24°B compared to grapes harvested lower than 24°B. Harvesting at an early stage of ripeness may also result in unstable phenolics as indicated by Canals *et al.* (2005) when investigating the influence of grape ripeness on the extraction of colour and phenolic compounds of Tempranillo grapes. In addition, in the study of Merrell *et al.* (2018) it was concluded that the type of tannin, composition and length, extracted during maceration was influenced by grape maturity which was also reported to be independent of the tannin concentration present in the grapes.

Polymeric pigment formation plays an important role in the sensory perception of a red wine (Cheynier *et al.*, 2006). A recent study reported by Merrell *et al.* (2018) showed that the initial anthocyanin concentration present in the berry skin is strongly correlated with polymeric pigment formation. Higher initial anthocyanin concentrations can therefore result in higher polymeric pigment formation that can contribute positively to the sensory perception of the wine. Grape anthocyanin levels can be modified by harvesting at different



maturity levels due to the fact that anthocyanin accumulation normally increases and continuous during ripening.

### 2.2.3 VITICULTURE PRACTICES

In a vineyard the physical characteristics vary, influencing anthocyanin and flavonoid content present in the berry (Teixera *et al.*, 2013). Anthocyanin and flavonol accumulation is affected by various environmental factors such as soil composition, temperature, light and vine water status (Downey *et al.*, 2006; Gonz  les-Neves *et al.*, 2012). The phenolic composition and accumulation in the berry can be modified by the application of different viticulture practices such as irrigation or leaf removal (Downey *et al.*, 2006). Although these practices can be applied to modify the phenolic content, the final phenolic composition is still highly variety as well as grapevine response dependent (Downey *et al.*, 2006).

There is currently significant evidence showing that climatic conditions influence phenolic concentration in grape berries. This statement is in agreement with the study of Gonz  les-Neves *et al.* (2012) who concluded that climate had a bigger effect on the anthocyanin content of Tannat wines than the winemaking practices applied in three different vintages. In another study the climate effect was shown in grapes originated from cooler areas that had higher levels of anthocyanins in the grapes compared to warmer areas (Nel *et al.*, 2014). Moreover, anthocyanin accumulation seems to be favoured by moderate temperatures (17-26  C) and might be inhibited at too high temperatures (>26   C) (Haselgrove *et al.*, 2000; Jackson and Lombard, 1993).

The effect of modifying the canopy microclimate on phenolic composition has been well investigated (Haselgrove *et al.*, 2000). Many practices have been applied to modify the phenolic composition of the grapes and to potentially improve the overall grape and wine quality (Chorti *et al.*, 2010; Dokoozlian and Hirschfeldt, 1995; Kennedy *et al.*, 2002). Leaf removal is a common canopy management practice applied to modify the phenolic composition of the grapes by exposing the berries to sunlight. Applying leaf removal at different phenological stages influences the phenolic profile of the grapes as has been reported in some studies (Teixera *et al.*, 2013). Open canopies generally result in grapes with a higher sugar level as well as improved acidity (Teixera *et al.*, 2013). Wine produced from sun-exposed berries has been observed with increased anthocyanin and flavonol content (Downey *et al.*, 2006; Mazza *et al.*, 1999; Yu *et al.*, 2016). Although moderate open canopies can be beneficial for optimal anthocyanin accumulation, full exposed grapes can be negatively affected since anthocyanin accumulation is inhibited at high temperatures (Haselgrove *et al.*, 2000).

Managing vine water status is an essential practice which can have a significant impact on the phenolic composition, while also influencing overall red wine quality (Matthews *et al.*, 1987; Nadal and Arola, 1995; Ojeda *et al.*, 2001; Zimman *et al.*, 2002). Studies have reported that optimum grapevine performance for agricultural purposes is not associated with large amounts of water, but rather with water deficit irrigation (Chaves *et al.*, 2007). The phenological timing of application has also a great impact on the accumulation, concentration and composition of phenolic compounds (Gonz  les-Neves *et al.*, 2012;

Kennedy *et al.*, 2002; Matthews *et al.*, 1990). For example pre-*veraison* deficit irrigation increased the colour of grapes compared to post-*veraison* deficit in the study of Matthews and Anderson (1988). Anthocyanin composition differences were also observed between pre- and post-*veraison* deficit irrigation by Ollé *et al.* (2011). Moreover, anthocyanin accumulation is greatly affected by osmotic stress (Downey *et al.*, 2006). Anthocyanin content could therefore be modified by influencing the vine water status (Downey *et al.*, 2006). It is widely reported that wine produced from grapes under water deficit conditions have been observed to contain higher anthocyanin levels (Castellarin *et al.*, 2007; Kennedy *et al.*, 2002; Ollé *et al.*, 2011). On the other hand, only a few studies have reported increased skin-derived proanthocyanidin levels present in wine produced from grapes under water deficit irrigation conditions (Downey *et al.*, 2006; Kennedy *et al.*, 2002).

## 2.3 VARIABLES AND PRACTICES INFLUENCING PHENOLIC EXTRACTION DURING ALCOHOLIC FERMENTATION

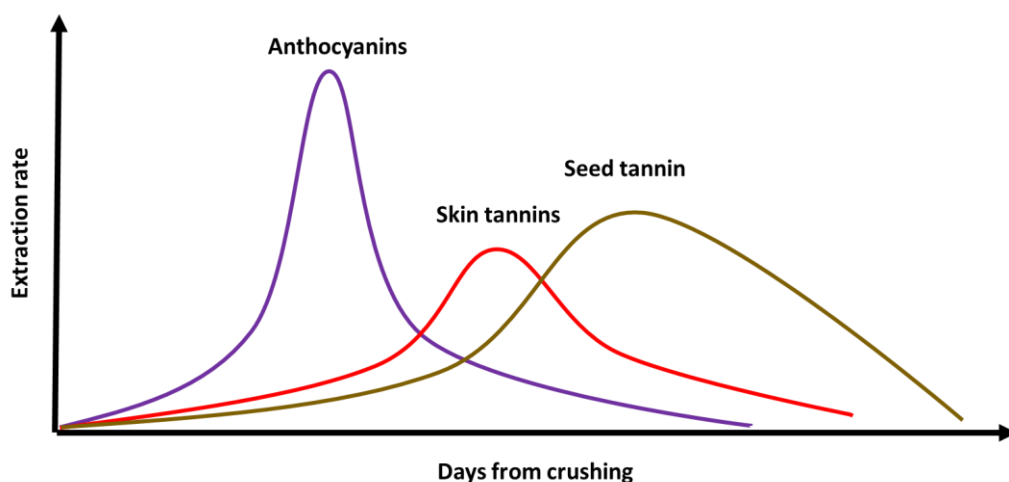
Different winemaking techniques and variables have been investigated to increase or modify phenolic extraction during maceration. Temperature appears as one of the most influential factors. Different methods relying on different temperatures can be applied, including cold soaking, must freezing, thermovinification and fermenting at high temperatures to modify phenolic extraction (Koyama *et al.*, 2007; Sacchi *et al.*, 2005). Other techniques and variables that have been reported to enhance extraction could be extended maceration, saignée and the usage of pectolytic enzymes (Sacchi *et al.*, 2005). Skin and juice mixing techniques were reported to also influence phenolic extraction (Sacchi *et al.*, 2005).

### 2.3.1 MACERATION LENGTH AND INCREASING ALCOHOL CONTENT

The maceration step refers to the winemaking period where phenolic compounds are extracted from the grape berry into the must and is performed by leaving the skins in contact with the fermenting must or wine over a certain period of time (Smith *et al.*, 2015). The effect of maceration is also influenced by temperature, skin contact time and grape composition which influence the final phenolic concentration of the wine (Gómez-Plaza *et al.*, 2001; Gonzáles-Neves *et al.*, 2012).

The maceration period can be divided into three different stages: pre-fermentative maceration (absence of alcohol), fermentative maceration (increase of alcohol concentration) and post-fermentative maceration (presence of high alcohol concentration).

**Figure 2.5** illustrates the effect of the maceration period on the extraction of anthocyanins, skin and seed-derived tannins.



**Figure 2.5:** Phenolic extraction during maceration. Adapted from Joscelyne, 2009.

As seen in **Figure 2.5**, during the first few days of maceration also known as pre-fermentative maceration, anthocyanins and skin proanthocyanidins will start to diffuse into the must after the grapes are crushed, due to their solubility in water and since the diffusion process is also independent of ethanol concentration and localization in the berry skin (González-Neves *et al.*, 2013; Ivanova *et al.*, 2012; Romero-Cascales *et al.*, 2005; Cheynier *et al.*, 1997). As maceration progresses anthocyanin extraction reaches a maximum during fermentative maceration, due to the adsorption-desorption equilibrium reached. A gradual decrease follows after an equilibrium has been reached until the end of the maceration period (Bautista-Ortín *et al.*, 2016; Canals *et al.*, 2005; Dimitrovska *et al.*, 2015; Gambuti *et al.*, 2009; Go and Lo, 1999; Harbertson *et al.*, 2009).

In addition, at the beginning of maceration different anthocyanin structures have different extraction rates due to their solubility in water (Cacho *et al.*, 2000). Malvidin is the most abundant anthocyanin present in both must and wine, followed by petunidin. However, during maceration a decrease of peonidin and cyanidin anthocyanin derived structures can occur (González-Neves *et al.*, 2008). Delphinidin, petunidin and cyanidin are more oxidizable anthocyanins compared to malvidin and peonidin and will decrease more rapidly in the wine (Cheynier *et al.*, 2006). The final concentration of malvidin is influenced by the co-occurrence of the other anthocyanins present in the wine during the winemaking process (García-Beneytez *et al.*, 2002). The anthocyanin concentration can decrease during fermentative maceration due to being reabsorbed by yeast cells, formation of polymeric pigments or degradation reactions (Cheynier *et al.*, 2006; Dimitrovska *et al.*, 2015; González-Neves *et al.*, 2012; Vazquez *et al.*, 2010). Interestingly, the polarity of the five glucoside anthocyanins also influences their adsorption by the yeast cell walls. Delphinidin is the most polar due to their amount of hydroxyl groups and will be adsorbed first, followed by cyanidin, petunidin, peonidin and malvidin (Cacho *et al.*, 2000; Medina *et al.*, 1997).

During fermentative maceration alcohol content increases as maceration progresses (Chittenden *et al.*, 2015; Sacchi *et al.*, 2005). An increase in alcohol content is favourable for seed extraction, since seed proanthocyanidins are located in the seed coat, covered with an outer lipid layer, isolating the seed and limiting extraction (Hernández-Jiménez *et al.*, 2012). Since seed proanthocyanidins are less soluble in water, an increase of alcohol content is important to extract seed proanthocyanidins from the seed as it helps to eliminate the lipidic

protective layer (Ivanova *et al.*, 2012). Interestingly, several studies reported seed proanthocyanidins were still extracted at low ethanol concentrations, however extraction occurs more rapidly at increased alcohol concentrations (Busse-Valverde *et al.*, 2010; Casassa *et al.*, 2009). In addition, extraction seems to be independent of the alcohol content present in the wine medium, once a specific level of seed cell hydration has been reached (Hernández-Jiménez *et al.*, 2012). On the other hand, authors have reported seed tannin extraction is mainly driven by the maceration length (skin contact time with the fermenting must). As seen in **Figure 2.5**, both skin and seed tannins have different diffusion kinetics during the maceration time when the fermenting must is in contact with the skins and seeds (Canals *et al.*, 2005). Seed tannin content will increase linearly compared to skin tannin content which will reach a plateau during the maceration (Cerpa-Calderón and Kennedy, 2008; Hernández-Jiménez *et al.*, 2012; Lerno *et al.*, 2015). Therefore, at the beginning of maceration the extraction of skin tannins will predominate the extraction of seed tannins (Hernández-Jiménez *et al.*, 2012; Koyama *et al.*, 2007). However, as maceration progresses, seed tannin extraction will overtake skin tannin extraction.

Due to different extraction kinetics, the final content of proanthocyanidins in the wine can be modified by managing the maceration length (Canals *et al.*, 2005; Casassa *et al.*, 2014; Harbertson *et al.*, 2009; Ivanova *et al.*, 2012; Sener *et al.*, 2012). Numerous studies have investigated the effect of the maceration length on phenolic extraction and final content. Cascales *et al.* (2005) reported anthocyanin extraction reached a maximum by day seven of maceration of Monastrell grapes and longer maceration time lead to a decrease of anthocyanin content. This is in agreement with other literature studies concluding anthocyanins reaches a maximum during maceration and will decrease as maceration progresses, due to reabsorption by yeast cells or solid parts, degradation and condensation reactions occurring during the winemaking process (Casassa *et al.*, 2009; Damijani *et al.*, 2011; Dimitrovska *et al.*, 2015; Gómez-Plaza *et al.*, 2001; Kelebek *et al.*, 2006; Koyama *et al.*, 2007; Sacchi *et al.*, 2005).

In the study of Gómez-Plaza *et al.* (2001) the maceration length had influenced the evolution of phenolics during aging. Increased total phenolic content was observed in the finished wines when longer maceration lengths were conducted compared to shorter maceration lengths (10 vs. 5 days of maceration). Additionally, Ivanova *et al.* (2012) reported similar results. Longer maceration lengths resulted in higher anthocyanins and tannin content present in aged wine compared to wine made from shorter maceration lengths. In addition, both reviews conducted by Sacchi *et al.* (2005) and Casassa *et al.* (2014) concluded that longer maceration with the skins in contact with the fermenting must, resulted in wines with increased extraction of seed tannins as well as increased formation of polymeric pigments. These results were observed and reported by numerous other studies as well (Casassa *et al.*, 2013; Casassa *et al.*, 2009; Gómez-Plaza *et al.*, 2001; Harbertson *et al.*, 2009; Romero-Cascales *et al.*, 2005; Zimman *et al.*, 2002). Polymeric pigment formation probably increased with longer maceration length, due to increased tannin extraction occurring as maceration progresses, resulting in increased condensation reactions occurring between anthocyanins and tannins forming pigmented polymers. Additionally, increased polymeric content can contribute to more stabilized red wine colour.

### 2.3.2 CAP MANAGEMENT

Cap management is a general technique applied in various cellars during red wine production. However, limited number of publications have reported the effect of cap management during fermentative maceration (Ichikawa *et al.*, 2012; Smith *et al.*, 2015).

It is well known during alcoholic fermentation a skin cap will start to form as fermentation progresses, due to carbon dioxide being produced, limiting contact between the skins and the juice. Contact between the skins, seeds and the juice are a crucial factor influencing extraction of phenolic compounds due to localization in the berry skin. Optimum contact would require frequent mixing of the cap formed, thereby limiting suboptimal extraction (Ichikawa *et al.*, 2012; Sacchi *et al.*, 2005).

Since phenolic extraction is a diffusion process, numerous juice/skin mixing techniques have been investigated to enhance phenolic extraction (De Beer *et al.*, 2006). General skin/juice mixing techniques applied in wineries include manually pushing the cap below the juice (i.e. punch down), mechanically spraying the juice over the cap (i.e. pump over) or mechanically mixing the cap with the liquid (i.e. rotary fermenter) several times a day (Smith *et al.*, 2015). Variable results have been reported when different mixing practices were compared to enhance phenolic extraction. In the review of Sacchi *et al.* (2005) high variation on the effect of the technique was reported dependent on the cultivar. In the study of Fischer *et al.* (2000) the different techniques (manual punch down, mechanical pump-over and punch down) were applied to mix the skins and juice of three different varieties and reported a different effect on phenolic content dependent on the variety. For example, mechanical punch down resulted in greater phenolic extraction compared to the other two methods for Pinot noir wine, whereas only small effects were noticed with the cultivar Portugieser. Additionally, the cultivar Dornfelder benefitted more from mechanical pump-overs compared to mechanical punch downs (Fischer *et al.*, 2000). However, it should be kept in mind that Pinot noir has a different phenolic profile compared to the other two cultivars and may have had different diffusion properties.

Another study compared the effect of manual punch down, pump over and rotary fermenters on phenolic extraction of Pinotage (Marais, 2003). The study concluded that the highest concentration of phenolics extracted when comparing the three different mixing techniques was rotary fermenters followed by punch down and pump-over. This is in agreement with the study of De Beer *et al.* (2006). The published research suggests that the phenolic composition of the wines was more dependent on grape variety, regardless of the mixing technique used.

In addition, one would expect enhanced mixing frequency (hourly compared to three hourly) would result in increased phenolic extraction, due to increased contact between the cap and the fermenting must. Chittenden *et al.* (2015) investigated the effect of punch down on the phenolic extraction of Merlot wine. It was concluded that two punch downs a day resulted in a wine with lower phenolic content compared to a wine made from no punch downs i.e. no

mixing of the cap. Similar results were concluded by Ichikawa *et al.* (2012). Punch down can lead to mechanical disruption of the cap, increasing adsorption, oxidation or precipitation of phenolic compounds. Additionally, extensive damaging of the grape skin with enhanced punch down frequencies may result in extraction of proanthocyanidin binding compounds, leading to adsorption or precipitation decreasing the proanthocyanidin content of the wine (Ichikawa *et al.*, 2012). Of course oxidation of the cap can also possibly lead to more unstable tannins that can result in a decrease of tannin extraction (Bosso *et al.*, 2011). However, evaluating the effect of mixing frequency applied on the phenolic content, no effect was observed on phenolic content (De Beer *et al.*, 2006; Marais, 2003).

Interestingly, various studies have been reported about the effect of different mixing techniques on the phenolic content of small scale vinifications. However, little research has been conducted on larger scale vinifications therefor, further research on commercial scale is necessary to confirm the effect of mechanical punch down on phenolic composition.

### 2.3.3 FERMENTATION TEMPERATURE

Temperature influences extraction of phenolic compounds during maceration, because it increases the degradation of berry tissues and increases diffusion of phenolics into the must (Koyama *et al.*, 2007; Nel *et al.*, 2014). Studies have shown that increases in temperature and ethanol content during alcoholic fermentation are positively correlated with the increased extraction of phenolic compounds (Hernández-Jiménez *et al.*, 2012; Nel *et al.*, 2014; Sacchi *et al.*, 2005). Anthocyanins are more easily extracted due to the increased permeability of the hypodermal cells with the increase of temperature (Sacchi *et al.*, 2005; Zimman *et al.*, 2002). Ough and Amerine (1959) reported that increased fermentation temperature lead to higher coloured wines in both Cabernet Sauvignon and Pinot noir wines. Damijani *et al.* (2011) reported maceration at high temperatures lead to a decrease of anthocyanin concentration. A decrease of anthocyanin concentration can be observed at high fermentation temperatures due to thermal degradation or adsorption reactions (Lerno *et al.*, 2015). An increase of temperature can also lead to an increase in polymeric pigment formation, resulting in greater coloured wine (Sacchi *et al.*, 2005).

The extraction of phenolics such as anthocyanins, polymeric pigments and tannins increases at higher fermentation temperatures due to enhanced permeability of the hypodermal cells, increasing anthocyanin levels as well enhanced solubility of phenolics such as tannins increases phenolic levels (Lerno *et al.*, 2015; Sacchi *et al.*, 2005; Sener *et al.*, 2012). In both studies of Girard *et al.* (1997, 2001) an increase of phenolic content was observed in Pinot noir wine fermented at higher temperatures. Increase of temperature might also increase tannin extraction (Smith *et al.*, 2015; Zimman *et al.*, 2002). Although an increase in phenolic content was observed in both studies, one should keep in mind that Pinot noir is a very different cultivar in terms of phenolic content and composition in comparison with for example Cabernet Sauvignon.

In addition, several studies have investigated the importance of increased fermentation temperature of the must on phenolic extraction (Damijani *et al.*, 2011; Fischer *et al.*, 2000;



Koyama *et al.*, 2007). The temperature of the must increases during alcoholic fermentation in combination with a cap starting to form which also promotes a thermal gradient between the must and the cap (Sacchi *et al.*, 2005). The importance of must temperature on phenolic extraction have been highlighted in numerous studies, however the influence of the thermal gradient, limiting contact between the skins, seeds and juice may also possibly influence phenolic extraction. In the study of Lerno *et al.* (2015) the effect of the thermal gradient between the must and cap was investigated. The study concluded that the must temperature had the greatest effect on phenolic extraction. The temperature of the must had an effect on the extraction rate of both skin and seed-derived compounds, but also on the final concentration of seed-derived phenolics present. Moreover, cap management techniques should play a key role in creating a homogeneous temperature in the wine medium, limiting a thermal gradient forming between the must and the cap, but also creating favourable conditions for enhanced phenolic extraction.

### 2.3.4 MACERATING ENZYMES

Anthocyanin extraction is limited due to their localization in the cell wall and can be trapped in the cell itself if the membrane is not properly ruptured during the maceration process (Sacchi *et al.*, 2005). Macerating enzymes is a winemaking practice that is conducted to rupture the skin cell walls and to increase extraction of phenolic compounds like anthocyanins and proanthocyanidins located in the vacuole of the berry skin (Hanlin *et al.*, 2009; Vazquez *et al.*, 2010). The effect of macerating enzymes on the colour of wine is contradictory. Pectolytic enzymes degrade the cell wall to release the pigments and increase the colour of a wine, but the purity of the enzyme has been named as an important factor. If the enzyme preparation contains  $\beta$ -glucosidases it can convert anthocyanins to a less stable forms resulting in colour loss rather than increased wine colour (Bautista-Ortín *et al.*, 2005). In some studies an increase of colour intensity was observed (Kelebek *et al.*, 2009; Nel *et al.*, 2014) while no effect was reported in other (Zimman *et al.*, 2002).

Macerating enzymes have also been investigated for their effect on the proanthocyanidin profile of wine and different results have been obtained. In some studies macerating enzymes increased the proanthocyanidin content of the wine (Busse-Valverde *et al.*, 2011; Kelebek *et al.*, 2009; Zimman *et al.*, 2002) while in other studies no effect was observed (Busse-Valverde *et al.*, 2010). In the study of Moreno-Pérez *et al.* (2012) macerating enzymes had a contradictory effect on the proanthocyanidin profile dependent of where the grapes originated from. In the review of Sacchi *et al.* (2005) it was concluded that pectinases do not increase anthocyanin extraction but increases the extraction of other phenolics compounds.

### 2.3.5 GRAPE COMPOSITION

Anthocyanin extraction is influenced by grape maturity, grape variety, cell wall composition and structure (Ortega-Regules *et al.*, 2006; Bautista-Ortín *et al.*, 2016; Romero-Cascales *et al.*, 2005; Smith *et al.*, 2015). Highly coloured grapes do not always result in highly coloured

wine, because of different factors influencing the extractability of anthocyanins from the skin (Bautista-Ortín *et al.*, 2006). In some studies a strong and positive correlation have been observed for anthocyanin and colour content measured in grapes and in wine (Bindon *et al.*, 2014; du Toit and Visagie, 2012; Jensen *et al.*, 2008). Although many literature studies have concluded that anthocyanin extraction kinetics between different cultivars follow similar patterns, the final concentration of anthocyanin present in wine is still variety dependent (Bautista-Ortín *et al.*, 2016; González-Neves *et al.*, 2008).

Cell wall composition is dependent of grape variety and influences the rate and extend of anthocyanin extraction (Ortega-Regules *et al.*, 2006; Ortega-Regules *et al.*, 2008). The grape berry cell wall is composed of a primary and secondary cell wall as well as a middle lamella. The primary cell wall consists of 90% polysaccharides and 10% of structural proteins (Hanlin *et al.*, 2009). The secondary cell wall is described as being rigid and thick structured and consist mainly of cellulose (Vorwerk *et al.*, 2004). The middle lamella consists of mainly pectin compounds, which are necessary to rupture during maceration to allow for anthocyanin extraction. The cell wall composition therefore influences anthocyanin extraction and content finally present in red wine. Differences were observed in the cell wall composition of four red varieties (Cabernet Sauvignon, Syrah, Merlot and Monastrell) by Ortega-Regules *et al.* (2006) and possibly influenced by anthocyanin extractability. In another study, Cabernet Sauvignon grapes had significant higher anthocyanin extractability compared to the other cultivars (du Toit and Visagie, 2012). Anthocyanins have a high affinity to bind to the cell wall material as well, limiting anthocyanin extraction (Bindon *et al.*, 2014).

The final concentration of condensed tannins present in red wine is greatly influenced by grape variety and extraction kinetics (Bautista-Ortín *et al.*, 2016). Some studies have shown that wines are more dependent of variety than the winemaking practice used. Busse-Valverde *et al.* (2010) reported the proanthocyanidin profile of Syrah, Cabernet Sauvignon and Monastrell wines being more dependent of variety compared to the winemaking practices applied. Skin and seed tannins have different extraction kinetics due to localization, chemical structure and solubility properties (Bautista-Ortín *et al.*, 2016). Measuring the hydrophobicity of proanthocyanidins gives information about the solubility and length of the polymer (Merrell *et al.*, 2018) and could be used to understand the extraction kinetics of the different proanthocyanidins later found in wines. Proanthocyanidin extraction is influenced by the hydrophobicity and number of hydroxyl residues present in skin and seed proanthocyanidins (Koyama *et al.*, 2007). Proanthocyanidins with a low mDP, low hydrophobicity and hydroxyl residues will be extracted earlier than those with higher mDP, hydrophobicity and hydroxyl residues during the maceration period (Koyama *et al.*, 2007).

The extractability of both skin and seed proanthocyanidins seems to be also influenced by the different tissue structure of the berry skins and seeds (Bindon *et al.*, 2010; Ichikawa *et al.*, 2012). The extractability of tannins from the different tissues was reported to be 54% from the skin, 30% from the seed and 15% are extracted from the flesh (Bindon *et al.*, 2010). Cell wall composition and structure determines the potential for condensed tannins to



bind to the cell wall material to form complexes. Proanthocyanidins have the highest affinity to bind to flesh cell wall material, with 47% of seed proanthocyanidin and 57% of skin proanthocyanidin binding to flesh cell wall material (Bindon *et al.*, 2010). Skin proanthocyanidins with higher molecular mass, have a higher affinity to bind to flesh cell wall material. This was not the case for skin cell wall material. Less proanthocyanidins interacted with skin cell wall material. These differences in affinity for these materials may occur due to cell wall material composition differences between flesh and skin cell walls (Bindon *et al.*, 2010). The structure of skin cell wall material may possibly limit the affinity of high molecular mass proanthocyanidins to bind to skin cell wall material. In addition, tannins can bind to several other compounds such as proteins, anthocyanins or polysaccharides during vinification, through both hydrophilic and hydrophobic interactions, forming complexes with these compounds and reducing the tannin content present in the wine (Vidal *et al.*, 2004). However, authors have reported tannins mainly form complexes with cell wall polysaccharides through hydrophobic interactions (Bindon *et al.*, 2010; Hanlin *et al.*, 2009).

## 2.4 QUANTIFICATION OF PHENOLIC COMPOUNDS DURING ALCOHOLIC FERMENTATION

A suitable, time-efficient, rapid and accurate method to quantify phenolic content during the fermentation process seems of importance, since phenolic compounds play a key role in red wine quality and can be modified during the winemaking process (Sacchi *et al.*, 2005). A number of spectrophotometric based methods to quantify phenolics have been reported, due to the ease, reliability and rapidness of these methodologies (Aleixandre-Tudo *et al.*, 2017). However, monitoring the extraction and evolution of phenolic compounds during the fermentation process requires an even more suitable, rapid and cost-effective approach. The use of spectroscopy calibrations in combination with chemometrics tools to quantify phenolics has been highlighted in a number of studies. A lot of research has been conducted to quantify phenolic content of finished wine, however measuring phenolic levels during red wine fermentations will allow winemakers to modify and monitor phenolic content while the process is taking place (Cozzolino, 2015). In light of this, spectroscopy applications have been attempted to quantify phenolic levels in fermenting wine samples (Aleixandre-Tudo *et al.*, 2018; Damberg *et al.*, 2012). In a recent study, tannin content of fermenting samples through PLS and MLR regression calibrations making use of UV spectroscopy and methyl cellulose precipitable tannin assay (MCP) as reference methods have been successfully reported (Damberg *et al.*, 2012). More recently, Aleixandre-Tudo *et al.* (2018) reported UV-Visible PLS prediction models for four major phenolic parameters during fermentation: MCP tannins, total anthocyanin content, colour density and total phenolics index using the reference methods reported by Glories, (1984), Iland, (2000) and Sarneckis *et al.* (2006). In addition, PLS calibrations also making use of UV-Vis spectral properties were successfully attempted to quantify 27 individual phenolic compounds obtained with HPLC (Aleixandre-Tudo *et al.*, 2018). Taking into account the rapidness, accuracy, affordability, ease and multiparametric nature (a single spectral measurement is needed to quantify multiple parameters) of UV-Visible spectroscopy applications, the use of

spectroscopy calibrations to monitor phenolic extraction during red wine fermentations seems thus to be highly suitable.

## **2.4.1 REFERENCE METHODS FOR PHENOLIC ANALYSIS**

### **2.4.1.1 QUANTIFICATION OF WINE COLOUR**

Since wine colour is the most visible quality parameter of red wine, quantification of colour is important. The most common method reported to estimate wine colour highlights the colour density method as preferred choice. Measuring the colour density at 420, 520 and 620 nm respectively represents the yellow, red and blue colourations observed in wine (Glories, 1984b; Sudraud, 1958). In addition, the wine colour hue, also known as tone, is the ratio of yellow-brown pigments to red pigments (420 nm)/ (520 nm) indicating the change occurring during aging from a more red coloured wine to an orange/brick coloured wine (Glories, 1984a; Glories, 1984b; Sudraud, 1958). On the other hand, another method known as CIELab colour space, is a colorimetric method used to measure the colour of wine, as perceived by consumers, over the visible range of the electromagnetic spectrum (CIE, 1978). This colorimetric method reports the colour density, tonality and luminosity of the wine (CIE, 1978). In addition, this method is also suitable to determine colour differences observed between wine samples (Martínez *et al.*, 2001).

### **2.4.1.2 QUANTIFICATION OF ANTHOCYANINS**

There are many methods that can be applied to quantify the total anthocyanin content of a wine sample as highlighted by the review article of Aleixandre-Tudo *et al.* (2017). Anthocyanins are observed in the visible spectra from 490-550 nm range and therefore easy to quantify regardless of other phenolic compounds that are present in the wine (Giusti *et al.*, 1996). Quantifying the total anthocyanin content, by diluting the wine sample with hydrochloric acid (HCl), have been reported as a simpler and shorter method compared to other methods, namely bisulphite bleaching and pH differential method (Iland, 2000; Mazza *et al.*, 1999; Ribéreau-Gayon and Stonestreet, 1965). The effect of bisulphite bleaching and pH adjustment on the anthocyanin form is well known (Ribéreau-Gayon *et al.*, 2006). Both methods quantify the anthocyanin content by modifying two of its chemical properties. On the other hand, another method known as the co-pigmentation assay is based on anthocyanin associations with other colourless substances in the wine matrix. This method includes co-pigmented anthocyanins in the colour measurement to determine an accurate estimation of the wine colour. In addition to these methods a modified Somers assay have been reported to quantify total anthocyanin content, colour density and hue, bisulphite resistant pigments as well as total phenolics (Mercurio *et al.*, 2007). This method relies on the effect of bisulphite bleaching and pH adjustment by addition of bisulphite, hydrochloric acid and acetaldehyde to the wine samples (Mercurio *et al.*, 2007).

### **2.4.1.3 QUANTIFICATION OF TOTAL PHENOLS**

All of the phenolic compounds have a characteristic phenol ring that has the ability to absorb light in the ultraviolet region (Harbertson and Spayd, 2006). The characteristic peak at 280 nm is defined as the representative wavelength to quantify the total phenolic content of the wine (Somers and Evans, 1977). The total phenolics index (TPI) is an easy and rapid method to calculate and quantify the total phenolic compounds present in the wine. Although the ultraviolet absorbance values is independent of the pH of the wine to quantify phenolic compounds, other non-phenolic compounds with a phenol ring can also be absorbed and result in inaccurate values presenting the total phenolic index of a wine (Harbertson and Spayd, 2006; Somers, 1998). Somers and Evans (1974) estimated an average of 4 units representing non-phenolic compounds being absorbed to be subtracted from the ( $A_{280}$ ) value for a more accurate and representative reading of the total phenolic index. Another method has been reported to quantify total phenolics as well, namely, the Folin-Ciocalteu index (FCI). This method is based on a redox reaction of phenolic compounds with an acidic reagent, known as the Folin-Ciocalteu reagent (FCR), in an alkaline medium (Singleton and Rossi, 1965). This redox reaction results in a blue-coloured complex quantified at 750 nm.

#### **2.4.1.4 QUANTIFICATION OF CONDENSED TANNINS OR PROANTHOCYANIDINS**

There are various methods available to quantify tannin content in grapes and wine. Methyl cellulose precipitable tannin assay (MCP) and bovine serum albumin (BSA) are well known tannin precipitation methods (Adams and Harbertson, 1999; Hagerman and Butler, 1978; Harbertson *et al.*, 2002; Sarneckis *et al.*, 2006). These methods are based on a polymer-tannin interaction that will result in an insoluble complex, removing the tannin content out of the solution by precipitation. MCP samples are recorded at 280 nm with values represented as mg/L epicatechin equivalents (Sarneckis *et al.*, 2006). BSA samples are quantified at 510 nm representing total tannin content after a colorimetric reaction with ferric chloride. Ribéreau-Gayon and Stonestreet, (1965) suggested an acid hydrolysis spectrophotometric method to quantify the total content of proanthocyanidins at 550 nm reported as grams per litre (g/L). Both precipitation-based methods were proved suitable to be used as reference methods to build prediction models and quantify tannin content (Aleixandre-Tudo *et al.*, 2015).

#### **2.4.1.5 QUANTIFICATION OF INDIVIDUAL PHENOLICS**

##### High performance liquid chromatography (HPLC)

Despite the above-mentioned methods being suitable for routine phenolic analysis, they only provide an estimation of the total content of a particular group of compounds. Moreover, the major criticism they receive is their lack of specificity. On the other hand, HPLC can be used to quantify individual phenolic compounds and estimate pigmented polymers (Peng *et al.*, 2002).

HPLC analysis entails the use of a specific column which phenolic compounds will pass through and separate under specific gradient conditions. Using an UV-Visible detector, individual phenolic compounds can be quantified after elution based on their retention times and spectral properties. The signal of the UV-Visible detector will result in a single peak on the chromatogram, followed by integration and quantification of the peak area (Peng *et al.*, 2002). The phenolic compounds are quantified by using calibration curves available for the individual phenolic compounds (Bakker *et al.*, 1986). However, external calibration curves are built for compounds whose standards are not available and may also be used to quantify phenolics that correspond to the same phenolic family. Monomeric compounds like anthocyanins and flavanols will elute easier without interference resulting in single peaks that will be quantified. Pigmented polymers elute over a longer period of time and due to coelutions occurring during elution, identification and quantification is therefore difficult. Peng *et al.* (2002) confirmed that a later peak eluting at the end of the chromatogram was characteristic of polymeric pigments concluding that the specific peak had longer retention time and a higher absorbance ratio compared to the monomeric peaks which would be expected from polymeric pigments. Several studies have reported HPLC as a suitable method to quantify individual phenolic compounds (Cerpa-Calderón and Kennedy, 2008; Gambuti *et al.*, 2009; Lerno *et al.*, 2015; Mazza *et al.*, 1999; Revilla *et al.*, 2016).

## 2.5 STATISTICAL ANALYSIS USED FOR WINE-RELATED DATA

Multivariate data analysis (MVDA) is a helpful tool to improve, understand and interpret complex data sets containing multiple variables, samples and time points. MVDA is used to build models and understand possible correlations in the data. There are numerous multivariate methods that have been applied in wine studies to extract information from data such as PCA, OPLS and PLS models, to name a few of them. The importance of a suitable multivariate approach to monitor red wine fermentation processes is thus a necessity. Batch statistical process control (BSPC) is a multivariate approach that can be used to monitor phenolic extraction in real time or evaluate the fermentation process after process completion. This approach seems thus suitable to monitor phenolic extraction kinetics data during maceration.

### 2.5.1 BATCH STATISTICAL PROCESS CONTROL (BSPC)

BSPC (Batch statistical process control) is a statistical approach which includes three matrices, namely a time component, variables and batches. This multivariate approach provides an overview of a batch process from initialization until completion (Eriksson *et al.*, 2013). It is a method that has been applied in different industrial processes to monitor batch evolution i.e. monitoring beer fermentations as well as Baker's yeast production (Andersen and Runger, 2011; García-Muñoz *et al.*, 2004; Kourti, 2003). The application of such a multivariate approach is important to monitor batch behaviour and evolution. Interestingly, as far as we know, only a limited number of studies have used this approach before and therefore the method still needs validation (Dahl *et al.*, 1999; Wold *et al.*, 1998). BSPC would thus be an interesting approach to monitor and evaluate red wine fermentations as

well as have a better understanding of phenolic extraction and evolution of different phenolic grape batches during the maceration process.

BSPC can be divided in two levels known as batch evolution modelling (BEM) followed by batch level modelling (BLM) (Eriksson *et al.*, 2013). BEM provides an overview of batch processing and batch development which is time dependent. Batch process models are based on the individual measurements of well-behaved batches to monitor, evaluate and predict the behaviour of new batches (Eriksson *et al.*, 2013). BEM can be used as a monitoring tool to monitor e.g. the progression of phenolic extraction during maceration. This approach would enable a winemaker to adapt the winemaking protocol or correct deviating batches during the fermentation process, modifying the phenolic content to a preferred wine style. In addition, BEM can also be used after process completion as well to evaluate behaviour within the batch during the process. On the other hand, BLM models can be built after process completion, with the aim to evaluate the performance of a single batch and compare it with other batches. PCA, PLS or OPLS may be used to present the model.

Principal component analysis also known as PCA provides an overview or summary of the collected data and determine the relationship between the observations and variables of the data set (Eriksson *et al.*, 2013). Observations in a data set can refer to chemical compounds or analytical samples for example. The variables are used to characterize the observations and can refer to spectral data or chromatographic data (HPLC) (Eriksson *et al.*, 2013). Building a PCA model is a common statistical technique used in the wine industry to explain the correlation and effect of different practices applied on the final wine profile (Casassa *et al.*, 2016; Girard *et al.*, 2001). In addition, partial least square (PLS) is used to inter-correlate two data sets (X & Y) by a linear multivariate model (Eriksson *et al.*, 2013). This is also an applicable method to wine-related data. For example in the study of Casassa *et al.* (2013) a PLSR to determine the correlation between the chemical (X predictor) and sensory data (Y response) with two latent factors (alcohol concentration and maceration effect) was conducted. Chemical variables were used in the regression model to predict the sensory descriptors of the wine and were separated by two factors (i.e. alcohol content and maceration effect). On the other hand, OPLS (Orthogonal PLS) is a modification of the PLS statistical technique which also correlates the relationship between two data sets (X & Y) but also explains the variation of data set X that is not correlated to dataset Y (Eriksson *et al.*, 2013). OPLS-DA is a discriminant statistical analysis approach to separate data based on allocated classes i.e. drives separation between data points to form classes or groups (Worley and Powers, 2016). Although OPLS-DA is a supervise approach, it is a suitable modelling tool to better understand separation between data points (classes). Nevertheless, OPLS-DA models may lead to unreliable conclusions and needs to be validated. Overall, all three mentioned models i.e. PCA, PLS, OPLS are suitable techniques to present multivariate data of batch nature dependent of variables and batches.

## 2.6 CONCLUSION

Due to phenolic compounds playing a key role in red wine quality, quantification of these compounds using reliable, rapid and cost-effective methods can be considered as an

important priority for wine industry practitioners as well as scientists. As a result, numerous studies to improve our current knowledge about phenolic extraction during maceration have been attempted. However, as far as we know, monitoring phenolic extraction during the different stages of maceration of various grape batches, with a wide range of phenolic content evaluated, have not been previously investigated in South Africa. Limitations in capacity for the analyses and statistical analyses might have contributed to this. Since phenolic compound extraction is a diffusion process, general red wine production is conducted with skin contact during maceration. Numerous authors have reported the importance of skin contact time during red wine production and noted the maceration length as the most crucial factor influencing phenolic content. Investigating the influence of skin and seed presence and absence on phenolic content of various grape batches could contribute to a better understanding of phenolic compound extraction during maceration, but also enable winemakers to possibly modify phenolic content during the maceration step to alternatively improve overall red wine quality.

In addition, authors have reported the lack of research done on the effect of cap management on phenolic content and results obtained investigating the effect of different cap mixing techniques were not clear. Studies have reported phenolic content were more variety dependent, regardless of cap mixing technique applied. However, authors have indicated the importance of skin contact between the skins, seeds and juice to enhance phenolic extraction. However, the important effect of must temperature on phenolic extraction have been highlighted in the study of Lerno *et al.* (2015) who investigated the effect of the thermal gradient forming between the cap and the must during alcoholic fermentation on phenolic content.

On the other hand, the extraction and evolution of these phenolic compounds during red wine production can be considered as a continuous process. A multivariate approach will be a more suitable method to analyse a large data set generated in such a study. Quantifying phenolic levels of fermenting samples with PLS calibrations using UV-Visible spectroscopy can be a valuable tool to overcome the complexity of testing a large number of phenolics in a large number of samples. In addition, data can be processed and presented with the help of batch statistical process control (BSPC) to monitor phenolic extraction under different maceration conditions (i.e. absence/presence of skins) providing an overview of batch behaviour and evolution within and between grape batches.

## 2.7 LITERATURE CITED

- Adams, D. O. (2006). Phenolics and ripening in grape berries. *Am. J. Enol. Vitic.*, 3, 249-256.
- Adams, D. O., and Harbertson, J. F. (1999). Use of alkaline phosphatase for the analysis of tannins in grapes and red wines. *Am. J. Enol. Vitic.*, 50, 247-252.
- Aleixandre-Tudo, J. L., Buica, A., Nieuwoudt, H. and du Toit, W. J. (2017). Spectrophotometric analysis of phenolic compounds in grapes and wines. *J. Agric. Food Chem.*, 65(20), 4009-4026.
- Aleixandre-Tudo, J. L., Nieuwoudt, H. and du Toit, W. J. (2018). Chemometric compositional analysis of phenolic compounds in fermenting samples and wines using different infrared spectroscopy techniques. *Talanta*, 176, 526-536.
- Aleixandre-Tudo, J. L., Nieuwoudt, H. and du Toit, W. J. (2015). Robust ultraviolet-visible (UV-vis) partial least-squares (PLS) models for tannin quantification in red wine. *J. Agric. Food Chem.*,



63, 1088-1098.

- Aleixandre-Tudo, J. L., Nieuwoudt, H., Olivieri, A. and du Toit, W. J. (2018). Phenolic profiling of grapes, fermenting samples and wines using UV- Visible spectroscopy with chemometrics. *Food Control*, 85, 11-22.
- Andersen, S. W. and Runger, G. C. (2011). Partitioned partial least squares regression with application to a batch fermentation process. *J. Chemom.*, 25, 159-168.
- Bakker, J., Preston, N. W. and Timberlake, C. F. (1986). The determination of anthocyanins in aging red wines: comparison of HPLC and spectral methods. *Am. J. Enol. Vitic.*, 37(2), 121-126.
- Bautista-Ortín, A. B., Busse-Valverde, N., Fernández-Fernández, J. I., Gómez-Plaza, E. and Gil-Muñoz, R. (2016). The extraction kinetics of anthocyanins and proanthocyanidins from grape to wine. *J. Int. Sci. Vigne Vin*, 50, 91-100.
- Bautista-Ortín, A. B., Fernández-Fernández, J. I., López-Roca, J. M. and Gómez-Plaza, E. (2006). The effect of grape ripening stage on red wine color. *J. Int. Sci. Vigne Vin.*, 40, 15-24.
- Bautista-Ortín, A. B., Martínez-Cutillas, A., Ros-García, J. M., López-Roca, J. M. and Gómez-Plaza, E. (2005). Improving colour extraction and stability in red wines: The use of maceration enzymes and enological tannins. *Int. J. Food Sci. Technol.*, 40, 867-878.
- Bindon, K. A., Madani, S. H., Pendleton, P., Smith, P. A. and Kennedy, J. A. (2014). Factors affecting skin tannin extractability in ripening grapes. *J. Agric. Food Chem.*, 62, 1130-1141.
- Bindon, K., Kassara, S., Hayasaka, Y., Schulkin, A. and Smith, P. (2014). Properties of wine polymeric pigments formed from anthocyanin and tannins differing in size distribution and subunit composition. *J. Agric. Food Chem.*, 62, 11582-11593.
- Bindon, K. A., Smith, P. A. and Kennedy, J.A. (2010) Interaction between grape-derived proanthocyanidins and cell wall material. 1. Effect on proanthocyanidin composition and molecular mass. *J. Agric. Food Chem*, 58, 2520-2528.
- Bindon, K. A., Smith, P. A., Holt, H. and Kennedy, J. A. (2010). Interaction between grape-derived proanthocyanidins and cell wall material. 2. Implications for vinification. *J. Agric. Food Chem.*, 58, 10736-10746.
- Bindon, K. A., Kassara, S., Cynkar, W. U., Robinson, E. M. C., Scrimgeour, N., Smith, P.A. (2014). Comparison of extraction protocols to determine differences in wine-extractable tannin and anthocyanin in *Vitis vinifera* L. cv. Shiraz and Cabernet Sauvignon grapes. *J. Agric. Food Chem.*, 62(20), 4558-4570.
- Bindon, K., Varela, C., Kennedy, J., Holt, H. and Herderich, M. (2013). Relationships between harvest time and wine composition in *Vitis vinifera* L. cv. Cabernet Sauvignon 1. Grape and wine chemistry. *Food Chem.*, 138, 1696-1705.
- Bosso, A., Panero, L., Petrozziello, M., Follis, R., Motta, S. and Guaita, M. (2011). Influence of submerged-cap vinification on polyphenolic composition and volatile compounds of Barbera wines. *Am. J. Enol. Vitic.*, 62(4), 503-511.
- Braidot, E., Zancani, M., Petrusa, E., Peresson, C., Bertolini, A., Patui, S., Macri, F. and Vianello, A. (2008). Transport and accumulation of flavonoids in grapevine (*Vitis vinifera* L.). *Plant Signal. Behav.*, 3(9), 626-632.
- Burin, V. M., Falcão, L. D., Gonzaga, L. V., Fett, R., Rosier, J. P. and Bordignon-Luiz, M. T. (2010). Colour, phenolic content and antioxidant activity of grape juice. *Ciênc. Technol. Aliment., Campinas*, 30(4), 1027-1032.
- Busse-Valverde, N., Gómez-Plaza, E., López-Roca, J. M., Gil-Muñoz, R., Fernández-Fernández, J. I. and Bautista-Ortín, A. B. (2010). Effect of different enological practices on skin and seed proanthocyanidins in three varietal wines. *J. Agric. Food Chem*, 58(21), 11333-11339.
- Busse-Valverde, N., Gómez-Plaza, E., López-Roca, J. M., Gil-Muñoz, R. and Bautista-Ortín, A. B. (2011). The extraction of anthocyanins and proanthocyanidins from grapes to wine during fermentative maceration is affected by the enological technique. *J. Agric. Food Chem.*, 59, 5450-5455.
- Cacho, J., Fernández, P., Ferreira, V. and Castells, J. E. (2000). Evolution of five anthocyanidin-3-glucosides in the skin of the Tempranillo, Moristel, and Garnacha grape varieties and influence of climatological variables. *Am. J. Enol. Vitic.*, 43(3), 244-248.
- Cadot, Y., Caillé, S., Samson, A., Barbeau, G. and Cheynier, V. (2012). Sensory representation of typicality of Cabernet franc wines related to phenolic composition: Impact of ripening stage and

- maceration time. *Anal. Chim. Acta.*, 732, 91-99.
- Canals, R., Llaudy, M. C., Vall, J., Canals, J. M. and Zamora, F. (2005). Influence of ethanol concentration on the extraction of color and phenolic compounds from the skin and seeds of Tempranillo grapes at different stages of ripening. *J. Agric. Food Chem.*, 53, 4019-4025.
- Casassa, L. F., Beaver, C. W., Mireles, M. S., Larsen, R. C., Hopfer, H., Heymann, H. and Harbertson, J. F. (2013). Influence of fruit maturity, maceration length, and ethanol amount on chemical and sensory properties of Merlot wines. *Am. J. Enol. Vitic.*, 64, 437-449.
- Casassa, L. F., Beaver, C. W., Mireles, M. S. and Harbertson, J. F. (2009). Effect of extended maceration and ethanol concentration on the extraction and evolution of phenolics, colour components and sensory attributes of Merlot wines. *Aus. J. Grape Wine Res.*, 19, 25-39.
- Casassa, L. F. and Harbertson, J. F. (2014). Extraction, evolution, and sensory impact of phenolic compounds during red wine maceration. *Annu. Rev. Food Sci. Technol.*, 5, 83-109.
- Casassa, L. F., Larsen, R. C. and Harbertson, J. F. (2016). Effects of vineyard and winemaking practices impacting berry size on evolution of phenolics during winemaking. *Am. J. Enol. Vitic.*, 67(3), 257-268.
- Castellarin, S. D., Matthews, M. A., Di Gaspero, G. and Gambetta, G. A. (2007). Water deficits accelerate ripening and induce changes in gene expression regulating flavonoid biosynthesis in grape berries. *Planta*, 227(1), 101-112.
- Cerpa-Calderón, F. K. and Kennedy, J. A. (2008). Berry integrity and extraction of skin and seed proanthocyanidins during red wine fermentation. *J. Agric. Food Chem.*, 56, 9006-9014.
- Chaves, M. M., Santos, T. P., Souza, C. R., Ortuño, M. F., Rodrigues, M. L., Lopes, C. M., Maroco, J.P. and Pereira, J. S. (2007). Deficit irrigation in grapevine improves water-use efficiency while controlling vigour and production quality. *Annal. Appl. Bio.*, 150(2), 237-252.
- Cheyrier, V., Salas, E., Souquet, J. M., Sarni-Manchado, P. Maury, C. and Fulcrand, H. (2006). Structure and properties of wine pigments and tannins. *Am. J. Enol. Vitic.*, 57(3), 298–305.
- Chittenden, R., Annand, M., King, P. and Russell, G. (2015). The effect of half plunging and no plunging as alternative winemaking techniques on phenolic extraction and pigment composition of wine. *S. Afr. J. Enol. Vitic.*, 36(1), 134-145.
- Chorti, E., Guidoni, S., Ferrandino, A. and Novello, V. (2010). Effect of different cluster sunlight exposure levels on ripening and anthocyanin accumulation in Nebbiolo grapes. *Am. J. Enol. Vitic.*, 61(1), 23-30.
- Commission Internationale de l'Eclairage. CIE Recommendations on Uniform Color Spaces, Color-Difference Equations, Psychometric Color Terms; CIE Public; Commission Internationale de l'Eclairage, 1978.
- Cozzolino, D. (2015). The role of visible and infrared spectroscopy combined with chemometrics to measure phenolic compounds in grape and wine samples. *Molecules*, 20, 726-737.
- Dahl, K. S., Piovoso, M. J. and Kosanovich, K. A. (1999). Translating third-order data analysis methods to chemical batch processes. *Chemom. Intell. Lab. Syst.*, 46(2), 161-180.
- Damberg, R. G., Mercurio, M. D., Kassara, S., Cozzolino, D. and Smith, P. A. (2012). Rapid measurement of methyl cellulose precipitable tannins using ultraviolet spectroscopy with chemometrics: Application to red wine and inter-laboratory calibration transfer. *Appl. Spectrosc.*, 66(6), 656-664.
- Damijanić, K., Staver, M., Ganić, K. K., Gracin, L., Bubola, M. and Palman, I. (2011). Effects of maceration temperature on the anthocyanins composition of cv. Teran wine. *Croat. J. Food Sci. Technol.*, 3(2), 16-25.
- De Beer, D., Joubert, E., Marais, J. and Manley, M. (2006). Maceration before and during fermentation: Effect on Pinotage wine phenolic composition, total antioxidant capacity and objective colour parameters. *S. Afr. J. Enol. Vitic.*, 27(2), 137-150.
- Dimitrovska, M., Bocevska, M., Dimitrovski, D. and Doneva-Sapceska, D. (2015). Evolution of anthocyanins during vinification of Merlot and Pinot Noir grapes to wine. *Acta Alimentaria*, 44(2), 259-267.
- Dokoozlian, N. K., and Hirschfeld, D. J. (1995). The influence of cluster thinning at various stages of fruit development on flame seedless table grapes. *Am. J. Enol. Vitic.*, 46, 429-436.
- Downey, M. O., Dokoozlian, N. K. and Krstic, M. P. (2006). Cultural practice and environmental



- impacts on the flavonoid composition of grapes and wine: a review of recent research. *Am. J. Enol. Vitic.*, 57(3), 257-268.
- du Toit, W. J. and Visagie, M. (2012). Correlations between South African red grape and wine colour and phenolic composition: Comparing the Glories, Iland and Bovine Serum Albumin tannin precipitation methods. *S. Afric. J. Enol. Vitic.*, 33, 33-41.
- Eriksson, L., Byrne, T., Johansson, E., Trygg, J. and Vikstrom, C. (2013). *Multi- and Megavariate Data Analysis Basic Principles and Applications*. Umetrics Academy, 3<sup>rd</sup> edn, Umeå, Sweden.
- Casassa, L. F., Beaver, C. W., Mireles, M. S. and Harbertson, J. F. (2013). Effect of extended maceration and ethanol concentration on the extraction and evolution of phenolics, colour components and sensory attributes of Merlot wines. *Aus. J. Grape Wine Res.*, 19(1), 25-39.
- Fischer, U., Strasser, M. and Gutzler, K. (2000). Impact of fermentation technology on the phenolic and volatile composition of German red wines. *Int. J. Food Sci. Technol.*, 35, 81-94.
- Flamini, R., Mattivi, F., De Rosso, M., Arapitsas, P. and Bavaresco, L. (2013). Advanced knowledge of three important classes of grape phenolics: Anthocyanins, stilbenes and flavonols. *Int. J. Mol. Sci.*, 14, 19651-19669.
- Fulcrand, H., Dueñas, M., Salas, E. and Cheynier, V. (2006). Phenolic reactions during winemaking and aging. *Am. J. Enol. Vitic.*, 57(3), 289-297.
- Gambutì, A., Capuano, R., Lecce, L., Fragasso, M. G. and Moio, L. (2009). Extraction of phenolic compounds from "Aglanico" and "Uva di Troia" grape skins and seeds in model solutions: Influence of ethanol and maceration time. *Vitis*, 48(4), 193-200.
- García-Beneytez, E., Revilla, E. and Cabello, F. (2002). Anthocyanin pattern of several red grape cultivars and wines made from them. *Eur. Food Res. Technol.*, 215, 32-37.
- García-Muñoz, S., Kourti, T. and MacGregor, J. F. (2004). Model predictive monitoring for batch processes. *Ind. Eng. Chem. Res.*, 43, 5929-5941.
- Garrido, J. and Borges, F. (2010). Wine and grape polyphenols - A chemical perspective. *Food Res. Int.*, 54, 1844-1858.
- Girard, B., Kopp, T. G., Reynolds, A. G. and Cliff, M. (1997). Influence of vinification treatments on aroma constituents and sensory descriptors of Pinot noir wines. *Am. J. Enol. Vitic.*, 48, 198-206.
- Girard, B., Yuksel, D., Cliff, M. A., Delaquis, P. and Reynolds, A. G. (2001). Vinification effects on the sensory, colour and GC profiles of Pinot noir wines from British Columbia. *Food Res. Int.*, 34, 483-499.
- Giusti, M. M. and Wrolstad, R. E. (1996). Radish anthocyanin extract as a natural red colorant for Maraschino cherries. *J. Food Sci.*, 61(4), 688-694.
- Glories, Y. (1984). *La couleur des vins rouges, 2eme partie. Connaissance de La Vigne et Du Vin*, 18, 253-271.
- Glories, Y. (1999). Substances responsible for astringency, bitterness and wine colour. *Journal International Des Sciences de La Vigne et Du Vin.*, 33, 107-110.
- Gil-Muñoz, R., Gómez-Plaza, E., Martínez, A. and López-Roca, J. M. (1999). Evolution of phenolic compounds during wine fermentation and post-fermentation: Influence of grape temperature. *J. Food. Comp. Anal.*, 12, 259-272.
- Gómez-Plaza, E., Gil-Muñoz, R., López-Roca, J. M., Martínez-Cutillas, A. and Fernández-Fernández, J. I. (2001). Phenolic compounds and color stability of red wines: Effect of skin maceration time. *Am. J. Enol. Vitic.*, 52(3), 266-270.
- González-Neves, G., Gil, G., Favre, G. and Ferrer, M. (2012). Influence of grape composition and winemaking on the anthocyanin composition of red wines of Tannat. *Int. J. Food Sci. Technol.*, 47, 900-909.
- González-Neves, G., Gil, G. and Barreiro, L. (2008). Influence of grape variety on the extraction of anthocyanins during the fermentation on skins. *Eur. Food. Res. Technol.*, 226, 1349-1355.
- González-Neves, G., Gil, G., Favre, G., Baldi, C., Hernández, N. and Traverso, S. (2013). Influence of winemaking procedure and grape variety on the colour and composition of young red wines. *S. Afr. J. Enol. Vitic.*, 34(1), 138-146.
- González-Neves, G., Favre, G., Gil, G., Ferrer, M. and Charamelo, D. (2015). Effect of cold pre-fermentative maceration on the color and composition of young red wines cv. Tannat. *J. Food*

Sci. Technol., 52(6), 3449-3457.

- Hagerman, A. E. and Butler, L. G. (1978). Protein precipitation method for the quantitative determination of tannins. J. Agric. Food Chem., 26, 809-812.
- Hanlin, R. L., Hrmova, M., Harbertson, J. F. and Downey, M. O. (2010). Review: Condensed tannin and grape cell wall interactions and their impact on tannin extractability into wine. Aus. J. Grape Wine Res., 16, 173-188.
- Harbertson, J. F., Kennedy, J. A. and Adams, D. O. (2002). Tannin in skins and seeds of Cabernet Sauvignon, Syrah, and Pinot noir berries during ripening. Am. J. Enol. Vitic., 53, 54-59.
- Harbertson, J. F., Mireles, M. S., Harwood, E. D., Weller, K. M. and Ross, C. F. (2009). Chemical and sensory effects of saignée, water addition, and extended maceration on high °Brix must. Am. J. Enol. Vitic. 60(4), 450-460.
- Harbertson, J.F. and Spayd, S. (2006). Measuring phenolics in the winery, Am. J. Enol. Vitic., 57(3), 280-288.
- Haselgrove, L., Botting, D., Van Heeswijck, R., Høj, P. B., Dry, P. R., Ford, C. and Iland, P. G. (2000). Canopy microclimate and berry composition: The effect of bunch exposure on the phenolic composition of *Vitis vinifera* L cv . Shiraz grape berries. Aus. J. Grape Wine Res., 6(2), 141-149.
- Herderich, M. J. and Smith, P. A. (2005). Analysis of grape and wine tannins: Methods, applications and challenges. Aus. J. Grape Wine Res., 11, 205-214.
- Hernández-Jiménez, A., Kennedy, J. A., Bautista-Ortín, A. B. and Gómez-Plaza, E. (2012). Effect of ethanol on grape seed proanthocyanidin extraction. Am. J. Enol. Vitic., 63(1), 57–61.
- Ichikawa, M., Ono, K., Matsudo, T. and Okuda, T. (2012). Effect of cap management technique on the concentration of proanthocyanidins in Muscat Bailey A wine. Food Sci. Technol. Res., 18(2), 201–207.
- Iland, P., Ewart, A., Sitters, J., Markides, A. and Bruer, N. (2000). Techniques for chemical analysis and quality monitoring during winemaking; Patrick Iland Wine Promotions: Campbelltown, South Australia.
- Ivanova, V., Vojnoski, B. and Stefova, M. (2012). Effect of winemaking treatment and wine aging on phenolic content in Vranec wines. Food Sci. Technol. Res., 49(2), 161-172.
- Jackson, D. I. and Lombard, P. B. (1993). Environmental and management practices affecting grape composition wine quality - A review. Am. J. Enol. Vitic., 44(4), 409-430.
- Jensen, J. S., Werge, H. H. M., Egebo, M. and Meyer, A. S. (2008). Effect of wine dilution on the reliability of tannin analysis by protein precipitation. Am. J. Enol. Vitic., 59, 103-105.
- Joscelyne, V. L. (2009). Consequences of extended maceration for red wine colour and phenolics. University of Adelaide, Australia.
- Kallithraka, S., Bakker, J. and Clifford, M. N. (2007). Evaluation of bitterness and astringency of (+)-catechin and (-)-epicatechin in red wine and in model solution. J. Sens. Stud., 12(1), 25-37.
- Kelebek, H., Canbas, A., Selli, S., Saucier, C., Jourdes, M. and Glories, Y. (2006). Influence of different maceration times on the anthocyanin composition of wines made from *Vitis vinifera* L. cvc. Boğazkere and Öküzgözü. J. Food Eng., 77, 1012-1017.
- Kelebek, H., Canbas, A. and Selli, S. (2009). Effects of different maceration times and pectolytic enzyme addition on the anthocyanin composition of *Vitis vinifera* cv. Kalecik karasi wines. J. Food Process. Preserv., 33, 296-311.
- Kennedy, J. A., Matthews, M. A. and Waterhouse, A. L. (2002). Effect of maturity and vine water status on grape skin and wine flavonoids. Am. J. Enol. Vitic., 53(4), 268-274.
- Kourti, T. (2003). Multivariate dynamic data modeling for analysis and statistical process control of batch processes, start-ups and grade transitions. J. Chemom., 17, 93-109.
- Koyama, K., Goto-yamamoto, N. and Hashizume, K. (2007). Influence of maceration temperature in red wine vinification on extraction of phenolics from berry skins and seeds of grape (*Vitis vinifera*). Biosci. Biotechnol. Biochem., 71, 958-965.
- Lerno, L., Reichwage, M., Ponangi, R., Hearne, L., Block, D. E. and Oberholster, A. (2015). Effects of cap and overall fermentation temperature on phenolic extraction in Cabernet Sauvignon fermentations. Am. J. Enol. Vitic., 66(4), 444-453.

- Lingua, M. S., Fabani, M. P., Wunderlin, D. A. and Baroni, M. V. (2016). From grape to wine: Changes in phenolic composition and its influence on antioxidant activity. *Food Chem.*, 208, 228-238.
- Ma, W., Guo, A., Zhang, Y., Wang, H., Liu, Y. and Li, H. (2014). A review on astringency and bitterness perception of tannins in wine. *Trends Food Sci. Technol.*, 40, 6-19.
- Marais, J. (2003). Effect of different wine-making techniques on the composition and quality of Pinotage wine. II. Juice/skin mixing practices. *S. Afri. J. Enol. Vitic.*, 24(2), 76-79.
- Martínez, J. A., Melgosa, M., Pérez, M. M., Hita, E. and Negueruela, A. I. (2001). Visual and instrumental color evaluation in red wines. *Food Sci. Technol. Int.*, 7(5), 439-444.
- Matthews, M. A. and Anderson, M. M. (1988). Fruit ripening in *Vitis vinifera* L.: Responses to seasonal water deficits. *Am. J. Enol. Vitic.*, 39(4), 313-320.
- Matthews, M. A., Ishii, R., Anderson, M. M. and O'Mahony, M. (1990). Dependence of wine sensory attributes on vine water status. *J. Sci. Food Agric.*, 51, 321-335.
- Matthews, M. A., Anderson, M. M. and Schultz, H. R. (1987). Phenologic and growth responses to early and late season water deficits in Cabernet franc. *Vitis*, 26(1), 147-160.
- Mattivi, F., Vrhovsek, U., Masuero, D. and Trainotti, D. (2009). Differences in the amount and structure of extractable skin and seed tannins amongst red grape varieties. *Aus. J. Grape Wine Res.*, 15, 27-35.
- Mazza, G., Fukumoto, L., Delaquis, P., Girard, B. and Ewert, B. (1999). Anthocyanins, phenolics, and color of Cabernet Franc, Merlot, and Pinot Noir wines from British Columbia. *J. Agric. Food Chem.*, 47(10), 4009-4017.
- Mcrae, J. M. and Kennedy, J. A. (2011). Wine and grape tannin interactions with salivary proteins and their impact on astringency: A review of current research. *Molecules*, 16(3), 2348-2364.
- Medina Rland, K., Boido, E., Dellacassa, E. and Carrau, F. (2005). Yeast interactions with anthocyanins during red wine fermentation. *Am. J. Enol. Vitic.*, 56(2), 104-109.
- Mercurio, M. D., Damberg, R. G., Herderich, M. J. and Smith, P. A. (2007). High throughput analysis of red wine and grape phenolics - Adaptation and validation of methyl cellulose precipitable tannin assay and modified somers color assay to a rapid 96 well plate format. *J. Agric. Food Chem.*, 55(12), 4651-4657.
- Merrell, C. P., Larsen, R. C. and Harbertson, J. F. (2018). Effects of berry maturity and wine alcohol on phenolic content during winemaking and aging. *Am. J. Enol. Vitic.*, 69(1), 1-11.
- Monagas, M., Bartolomé, B. and Gómez-Cordovés, C. (2005). Updated knowledge about the presence of phenolic compounds in wine. *Crit. Rev. Food Sci. Nutri.*, 45, 85-118.
- Monagas, M., Gómez-Cordovés, C., Bartolomé, B., Laureano, O. and Da Silva, J. M. R. (2003). Monomeric, oligomeric, and polymeric flavan-3-ol composition of wines and grapes from *Vitis vinifera* L. Cv. Graciano, Tempranillo, and Cabernet Sauvignon. *J. Agric. Food Chem.*, 51, 6475-6481.
- Monagas, M., Gómez, G. and Bartolomé, B. (2005). Evolution of polyphenols in red wines from *Vitis vinifera* L. during aging in the bottle. *Eur. Food. Res. Technol.*, 220, 607-614.
- Moreno-Pérez, A., Fernández-Fernández, J. I., Bautista-Ortín, A. B., Gómez-Plaza, E., Martínez-Cutillas, A. and Gil-Muñoz, R. (2013). Influence of winemaking techniques on proanthocyanidin extraction in Monastrell wines from four different areas. *Eur. Food. Res. Technol.*, 236, 473-481.
- Nadal, M. and Arola, L. (1995). Effects of limited irrigation on the composition of must and wine of Cabernet Sauvignon under semi-arid conditions. *Vitis*, 34(3), 151-154.
- Nel, A. P., van Rensburg, P. and Lambrechts, M. G. (2014). The influence of different winemaking techniques on the extraction of grape tannins and anthocyanins. *S. Afri. J. Enol. Vitic.*, 35(2), 304-320.
- Noble, A. C. (1998). Why do wines taste bitter and feel astringent? Waterhouse, A. L. and Ebeler, S. E. (eds). *Chemistry of wine flavour*. Oxford University Press, DC, USA, 156-165.
- Ojeda, H., Deloire, A. and Carbonneau, A. (2001). Influence of water deficits on grape berry growth. *Vitis*, 40(3), 141-145.
- Ollé, D., Guiraud, J. L., Souquet, J. M., Terrier, N., Ageorges, A., Cheynier, V. and Verries, C. (2011). Effect of pre- and post-veraison water deficit on proanthocyanidin and anthocyanin

- accumulation during Shiraz berry development. *Aus. J. Grape Wine Res.*, 17(1), 90-100.
- Ortega-Regules, A. , Romero-Cascales, I., Ros-García, J. M., López-Roca, J. M. and Gómez-Plaza, E. (2006). A first approach towards the relationship between grape skin cell-wall composition and anthocyanin extractability. *Anal. Chim. Acta*, 563, 26-32.
- Ortega-Regules, A., Ros-García, J. M., Bautista-Ortín, A. B., López-Roca, J. M. and Gómez-Plaza, E. (2008). Differences in morphology and composition of skin and pulp cell walls from grapes (*Vitis vinifera* L.): Technological implications. *Eur. Food Res. Technol.*, 227, 223-231.
- Ough, C. S. and Amerine, M. A. (1961). Studies on controlled fermentation. V. Effects on color, composition, and quality of red wines. *Am. J. Enol. Vitic.*, 12, 9-19.
- Peng, Z., Iland, P. G., Oberholster, A., Sefton. and Waters, E. J. (2002). Analysis of pigmented polymers in red wine by reverse phase HPLC. *Aus. J. Grape Wine Res.*, 8, 70-75.
- Petrovic, A., Jovic, S., Gojkovic, L. and Bursac, M. (2012). Influence of winemaking techniques on the resveratrol content, total phenolic content and antioxidant potential of red wines. *Food Chem.*, 131, 513-518.
- Revilla, E., Losada, M. and Gutiérrez, E. (2016). Phenolic composition and color of single cultivar young red wines made with Mencía and Alicante-Bouschet grapes in AOC Valdeorras (Galicia, NW Spain). *Beverages*, 2(3), 18.
- Ribéreau-Gayon, P., Glories, Y., Maujean, A. and Dubourdieu, D. (2006). Handbook of Enology: The microbiology of wine and vinifications. *Handbook of Enology*, 2.
- Ribéreau-Gayon, P. and Stonestreet, E. (1965). Le dosage des anthocyanes dans le vin rouge. *Bulletin de La Société Chimique de France*, 9, 2649-2652.
- Romero-Cascales, I., Fernández-Fernández, J. I., López-Roca, J. M. and Gómez-Plaza, E. (2005). The maceration process during winemaking extraction of anthocyanins from grape skins into wine. *Eur. Food. Res. Technol.*, 221, 163-167.
- Sacchi, K. L., Bisson, L. F. and Adams, D. O. (2005). A review of the effect of winemaking techniques on phenolic extraction in red wines. *Am. J. Enol. Vitic.*, 56(3), 197-206.
- Sarneckis, C. J., Damberg, R. G., Jones, P., Mercurio, M., Herderich, M. J. and Smith, P. A. (2006). Quantification of condensed tannins by precipitation with methyl cellulose: Development and validation of an optimised tool for grape and wine analysis. *Aus. J. Grape Wine Res.*, 12(1), 39-49.
- Segade, S. R., Vázquez, E. S. and Losada, E. D. (2008). Influence of ripeness grade on accumulation and extractability of grape skin anthocyanins in different cultivars. *J. Food Comp. Anal.*, 21(8), 599-607.
- Sener, H and Yildirim H. K. (2013). Influence of different maceration time and temperatures on total phenols, colour and sensory properties of Cabernet Sauvignon wines. *Food Sci Technol Int.*, 19(6), 523-533.
- Singleton, V. L. and Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am. J. Enol. Vitic.*, 16(3), 144-158.
- Smith, P. A., Mcrae, J. M. and Bindon, K. A. (2015). Impact of winemaking practices on the concentration and composition of tannins in red wine. *Aus. J. Grape Wine Res.*, 21, 601-614.
- Somers, C. (1998). The wine spectrum: An approach towards objective definition of wine quality. *Winetitles*, Adelaide.
- Somers, T. C. and Evans, M. E. (1974). Wine quality: Correlations with colour density and anthocyanin equilibria in a group of young red wines. *J. Sci. Food Agric.*, 25(11), 1369-1379.
- Somers, T. C. and Evans, M. E. (1977). Spectral evaluation of young red wines: Anthocyanin equilibria, total phenolics, free and molecular SO<sub>2</sub>, 'chemical age. *J. Sci. Food Agric.*, 28(3) 279-281.
- Cheyrier, V., Hidalgo Arellano, I., Souquet, J. M. and Moutounet, M. (1997). Estimation of the oxidative changes in phenolic compounds of Carignane during winemaking. *Am. J. Enol. Vitic.*, 48, 225-228.
- Teixera, A., Eiras-Dias, J., Castellarin, S. D. and Gerós, H. (2013). Berry phenolics of grapevine under challenging environments. *Int. J. Mol. Sci.*, 14, 18711-18739.
- Vázquez, E. S., Segade, S. R. and Fernández, I. O. (2010). Effect of the winemaking technique on phenolic composition and chromatic characteristics in young red wines. *Eur. Food. Res.*

Technol., 231, pp. 789–802.

- Vidal, S., Francis, L., Noble, A., Kwiatkowski, M., Cheynier, V. and Waters, E. (2004). Taste and mouth-feel properties of different types of tannin-like polyphenolic compounds and anthocyanins in wine. *Anal. Chim. Acta*, 513, 57-65.
- Vorwerk, S., Somerville, S. and Somerville, C. (2004). The role of plant cell wall polysaccharide composition in disease resistance. *Trends Plant Sci.*, 9(4), 203-209.
- Waterhouse, A. L. (2002). Wine phenolics. *Ann. N.Y. Acad. Sci.*, 957, 21-36.
- Wold, S., Kettaneh, N., Fridén, H. and Holmberg, A. (1998). Modelling and diagnostics of batch processes and analogous kinetic experiments. *Chemom. Intell. Lab. Syst.*, 44, 331-340.
- Worley, B. and Powers, R. (2016). PCA as a practical indicator of OPLS-DA model reliability. *Curr Metabolomics*, 4(2), 97-103.
- Yu, R., Cook, M. G., Yacco, R. S., Watrelot, A. A., Gambetta, G., Kennedy, J. A. and Kurtural, S. K. (2016). Effects of leaf removal and applied water on flavonoid accumulation in grapevine ( *Vitis vinifera* L. cv. Merlot) berry in a hot climate. *J. Agric. Food Chem.*, 64, 8118-8127.
- Zimman, A., Joslin, W., Lyon, M., Meier, J. and Waterhouse, A. (2002). Maceration variables affecting phenolic composition in commercial-scale Cabernet Sauvignon winemaking trials. *Am. J. Enol. Vitic.*, 53(2), 93-98.

# Chapter 3

---

## Research results

**The effect of different maceration conditions on the extraction of phenolic compounds - A batch statistical process control approach.**



### 3.1 INTRODUCTION

Phenolics are plant-derived compounds present in a variety of food and beverage products (Cerpa-Calderón and Kennedy, 2008; Teixeira *et al.*, 2013). Phenolic compounds in red wine have been investigated in several studies for their important role and contribution towards sensorial and chemical properties as well as possible health benefits (Koyama *et al.*, 2007; Harbertson *et al.*, 2009; Monagas *et al.*, 2017). Red wine is a complex beverage which consists of numerous chemical components including phenolic compounds, which influence its colour, flavour and mouthfeel properties (Cheynier *et al.*, 1997; Casassa and Harbertson, 2014). Phenolic compounds found in grapes and wine have been classified as non-flavonoids (hydroxycinnamic acids, hydroxybenzoic acids and stilbenes) and flavonoids (anthocyanins, flavan-3-ols and flavonols) (Downey *et al.*, 2006; Teixeira *et al.*, 2013; Lerno *et al.*, 2015). Anthocyanins are known as the red pigments located in the vacuoles of the berry skin which are responsible for red wine's colour. On the other hand, proanthocyanidins are located in both skins and seeds of grape tissue and contribute to wine structure and mouthfeel attributes (Monagas *et al.*, 2005; Kelebek *et al.*, 2006; González-Neves *et al.*, 2012; Hernández-Jiménez *et al.*, 2012). Since the desirable phenolic compounds are located in the berry skins and seeds, red wine fermentations are conducted with skin contact to enhance extraction of phenolic compounds (Bindon *et al.*, 2010; Bautista-Ortín *et al.*, 2016).

The influence of different winemaking practices on the phenolic profile of red wines have been investigated in several studies (Sacchi *et al.*, 2005; Casassa and Harbertson, 2014; Smith *et al.*, 2015). The extent of the maceration and the conditions during this period have a great influence on the extraction of phenolic compounds and subsequent reactions they are involved in, which also influences the sensorial properties of the wine (Kelebek *et al.*, 2006; Koyama *et al.*, 2007; González-Neves *et al.*, 2008; Vazquez *et al.*, 2010). During the maceration process the phenolic compounds diffuse from the skins and seeds to the must during red wine fermentation (Romero-Cascales *et al.*, 2005; Cheynier *et al.*, 2006; Nel *et al.*, 2014). Since maceration is a selective extraction process, the time of maceration combined with variables such as different ethanol levels and temperatures influence the rate and extent of diffusion of specific phenolic compounds (Gómez-Plaza *et al.*, 2001; Romero-Cascales *et al.*, 2005; Sacchi *et al.*, 2005; Koyama *et al.*, 2007; Smith *et al.*, 2015). Shorter maceration times will promote the extraction of skin-derived phenolic compounds such as anthocyanins and skin proanthocyanidins, due to their localization and increased solubility in water. However, seed-derived proanthocyanidins, which are more soluble in alcohol, will benefit from longer maceration periods (Gómez-Plaza *et al.*, 2001; Canals *et al.*, 2005; Ivanova *et al.*, 2012).

Contact between the solids and must is therefore a crucial factor to enhance extraction of desirable phenolic compounds (García-Beneytez *et al.*, 2002; Harbertson *et al.*, 2009; Nel *et al.*, 2014; Lerno *et al.*, 2015). Cap management is an important winemaking practice applied

to prevent oxidation and bacterial growth, while facilitating the contact time between the must and skins and seeds (Ichikawa *et al.*, 2012). Few studies have investigated different cap management techniques to increase extraction of phenolic compounds during red wine fermentations (Marais, 2003; Sacchi *et al.*, 2005; De Beer *et al.*, 2006; Ichikawa *et al.*, 2012). The results obtained (punch down, pump over, submerged cap) were strongly dependent of the variety itself (Fischer *et al.*, 2000; Chittenden *et al.*, 2015).

Monitoring the extraction of phenolic compounds during alcoholic fermentation is a useful technique to manage the phenolic profile of a wine. On the other hand, spectroscopy is a suitable approach that could be used to measure and monitor phenolic compounds during alcoholic fermentation, since it is a simple, cost effective and rapid procedure compared to other methods that are time consuming and expensive (Harbertson and Spayd, 2006; Ivanova *et al.*, 2012; Cozzolino, 2015). Phenolic compounds have different spectral properties with characteristic features dependent on the specific phenolic class (Harbertson and Spayd, 2006). Although each phenolic class has different absorption features, phenolic compounds have a characteristic phenol ring with the ability to absorb light in the ultraviolet (UV) region and are therefore suitable to be quantified with spectrophotometric measurements (Aleixandre-Tudo *et al.*, 2017). Some studies have reported the effectiveness of UV-Vis spectroscopy to monitor phenolic compounds during fermentation (Aleixandre-Tudo *et al.*, 2018; Aleixandre-Tudo and Du Toit, 2019). Additionally batch statistical process control (BSPC) is a multivariate approach used in various industries to monitor and evaluate batch evolution, since most industrial processes are of a batch type (Eriksson *et al.*, 2013). BSPC have been applied to monitor Baker's yeast production, pharma-, biopharma- and beer fermentations (Wold *et al.*, 1998; Eriksson *et al.*, 2013). Batch statistical process control can be a suitable method to monitor red wine fermentations, however, limited research is currently available on this topic (Aleixandre-Tudo and du Toit, 2019).

The aim of the study was thus to monitor and evaluate phenolic extraction kinetics of Cabernet Sauvignon and Shiraz grapes during alcoholic fermentation under different maceration conditions (skin contact length i.e. presence/absence of skins) and punch down strategies (low versus high frequency, performed at different times during fermentation). A combination of phenolic data obtained using PLS spectroscopy calibrations and BSPC (batch statistical process control) was evaluated to better understand phenolic extraction during the fermentation process under different maceration conditions.

## **3.2 MATERIALS AND METHODS**

### **3.2.1 REAGENTS**

Ethanol (96%) was obtained from Merck (Darmstadt, Germany). Sodium Hydroxide (0.333N) was obtained from Cameron Chemicals (Cape Town, South Africa). Potassium iodate (N/64) was purchased from Cameron Chemicals (Cape Town, South Africa). Reagent



used for UV-Vis spectrophotometric measurements (hydrochloric acid (HCl)) were obtained from Sigma-Aldrich Chemie (Steinheim, Germany).

### 3.2.2 EXPERIMENTAL DESIGN

The study was conducted with four Shiraz and four Cabernet Sauvignon grape batches sourced from different vineyard blocks located in the Western Cape, South Africa (**Table 3.2.1**). The grapes were harvested in 2017 ranging from 23-26 °Brix. The grapes of each batch (vineyard) were randomly divided into 12 crates, each containing 20 kg of grapes, at the experimental cellar of the Department of Viticulture and Oenology (University of Stellenbosch, South Africa). After the grapes were cooled in a 4 °C room, the 12 crates of each vineyard batch were randomly marked according to the experimental design shown in **Figure 3.2.1**. One crate of grapes was thus used per vinification. Berry sampling was then conducted by randomly selecting 100 berries from different clusters of each crate. Grapes were frozen at -20°C until analysed.

**Table 3.2.1:** Cabernet Sauvignon and Shiraz batches vineyard localization.

Batch name	Cultivar	Region in Western Cape	Block name
2	Cabernet Sauvignon	Stellenbosch	Faure
3	Shiraz	Darling/Swartland	Alexanderfontein/Nyani
4	Shiraz	Stellenbosch	Faure
5	Shiraz	Elgin	Valley Green
6	Cabernet Sauvignon	Stellenbosch	Helderbergkloof
7	Shiraz	Stellenbosch	Navarre
8	Cabernet Sauvignon	Stellenbosch	Skoonheid
9	Cabernet Sauvignon	Stellenbosch	Bilton

As shown in **Table 3.2.2** three different pressing times (1: skin maceration until 1/3<sup>rd</sup> of alcoholic fermentation, 2: skin maceration until 2/3<sup>rd</sup> of alcoholic fermentation, 3: skin maceration until the end of alcoholic fermentation) during alcoholic fermentation were investigated at two different levels of punch down (i.e. standard vs. increased punch down frequency). The treatments (12 punch downs per day, T) and controls (3 punch downs per day, C) of the three different pressing times were conducted in duplicate for each grape batch. Samples were collected twice a day (morning and afternoon) in 2 mL eppies after crushing and destemming until the end of fermentation. All the samples were collected and analysed on the same day.

**Table 3.2.2:** Experimental design.

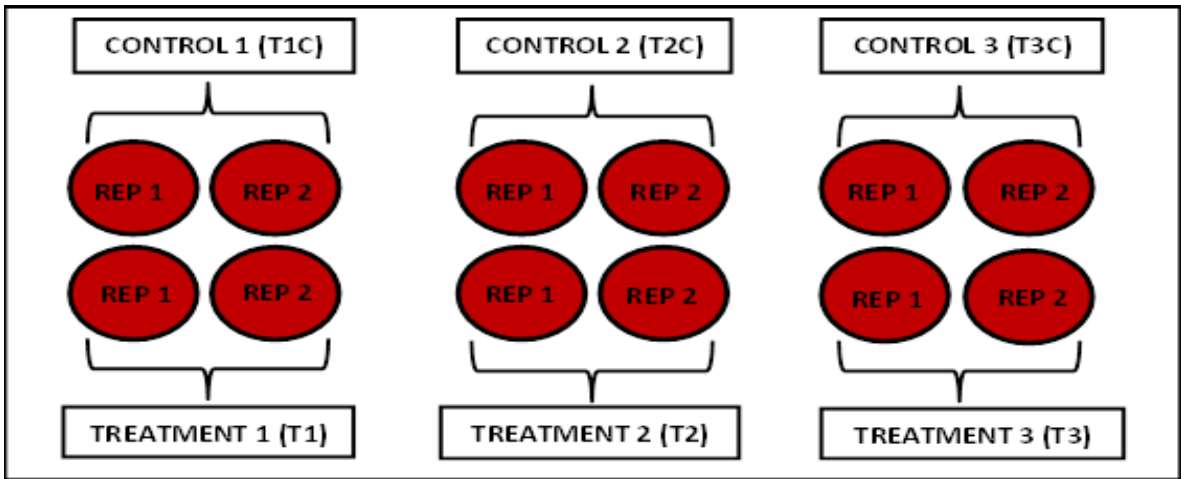
Pressing time	Skin contact length (i.e. presence/absence of skins)	Code
1	Skin maceration until 1/3 <sup>rd</sup> of alcoholic fermentation, skins pressed around 16°B. Punch downs occurred from the start of fermentation until 16°B.	T1 <sup>a</sup>
		C1 <sup>b</sup>
2	Skin maceration until 2/3 <sup>rd</sup> of alcoholic fermentation, skins pressed around 8°B. Punch downs occurred from 16°B to 8°B.	T2 <sup>a</sup>
		C2 <sup>b</sup>
3	Skin maceration until the end of alcoholic fermentation, skins pressed around 0°B. Punch downs occurred from 8°B to 0°B.	T3 <sup>a</sup>
		C3 <sup>b</sup>

T<sup>a</sup> Increased punch down frequency applied (12 punch downs per day)  
C<sup>b</sup> Standard punch down frequency applied (3 punch downs per day)

3.2.3 Winemaking

Twelve crates representing one grape batch were separately crushed and destemmed in 25 L plastic buckets following grape juice sampling for standard analysis (**Figure 3.2.1**). Fermentation took place at 25 °C in a temperature-controlled room. Grape juice analysis included standard measurement of soluble solids (°Balling), total titratable acidity (g/L) and pH. The grapes received 30 mg/L of sulphur dioxide (SO<sub>2</sub>) at crushing and were inoculated with 0.3 g/L commercial yeast strain Lalvin ICD D21 (*Saccharomyces cerevisiae*, Lallemant Inc., Montreal, Canada). Pectolytic enzyme (Lafase He Grand Cru, Laffort, Bordeaux, France) were also added to all the buckets following the manufacturer’s instructions. A yeast nutrient (0.25 g/L Fermaid K, Lalvin ICV D21, Lallemant Inc., Montreal, Canada) were added after 2-3 °Balling drop.

Alcoholic fermentation was completed in 25 L plastic buckets and finished wines pressed according to the experimental design (**Table 3.2.2**). All the vinifications were pressed in an open basket press and completed alcoholic fermentation in 20 L plastic buckets. After the completion of alcoholic fermentation, the wines were racked off into 4.5 L glass bottles for malolactic fermentation. The vinifications were inoculated with 0.01 g/L commercial lactic acid bacteria *Oenococcus oeni* VP 41 (Lalvin ICV D21, Lallemant Inc., Montreal, Canada). Malolactic fermentation was monitored through enzymatic analyses measuring malic acid levels of the fermenting samples weekly. Malolactic fermentation was completed in a 20°C room. The finished wines were racked off and 50 mg/L of SO<sub>2</sub> were added before the wines underwent cold stabilization at -4°C for three weeks. Subsequent to cold stabilization wines were racked off, followed by standard wine analysis, before bottling in 750 mL green glass bottles closed with screw tops. According to the results obtained from the standard wine analysis the SO<sub>2</sub> levels were adjusted to 45 mg/L of free SO<sub>2</sub>. The bottled wines were stored at 15°C.



**Figure 3.2.1:** Schematic layout of the experimental design after crushing and destemming in the 25 °C fermentation room of one batch.

### 3.2.4 ANALYSIS

#### Grapes

The method reported by Iland (2000) was used to extract and analyse phenolic compounds from the berry skin and seeds. The frozen berry samples of each fermentation were removed from the -20°C room, a day before sample preparation, to thaw the berries. Fifty berries were randomly selected per treatment and their weight recorded and homogenized separately in a 50 mL falcon tube with an Ultra-Turrax T25 homogenizer (Janke & Kunkel GmbH & Co., Germany) for 2 minutes at high speed. The homogenized samples were frozen at -20°C until analysis. Prior to analysis the frozen homogenates were removed to thaw. One gram of each homogenized sample was weighed in a 15 mL falcon and 10 mL of the extraction solvent (50% v/v aqueous ethanol solution adjusted to a pH of 2) added to the sample. The samples were placed in a Branson 5510 Lasec sonicator for one hour and manually shaken every 15 minutes. After one hour the samples were centrifuged in a 7366 Hermle centrifuge (Wehingen, Germany) at 5000 rpm for five minutes. In individual test tubes 100 µL of each sample were pipetted from the clarified supernatant and diluted with 1.9 mL of 1M HCl solution and placed in a dark cupboard followed by a waiting period of one hour. After one hour the samples were removed from the cupboard and 200 µL of each sample were pipetted into a UV-Visible Nunc F96 MicroWell plate (Nunc, Langenselbold, Germany) and placed in a Multiskan GO Microplate Spectrophotometer. Four phenolic parameters (colour density, anthocyanin content, tannin concentration and total phenols) were quantified based on the Ultraviolet-visible (UV-VIS) spectroscopy data collected with the Multiskan GO Microplate Spectrophotometer (Thermo Fisher Scientific, Inc., Waltham, MA, USA) using prediction PLS calibrations as reported elsewhere (Aleixandre-Tudo *et al.*, 2018).

#### Must and wine phenolic analysis

For the samples collected during alcoholic fermentation, phenolic levels were quantified through PLS calibrations (Aleixandre-Tudo *et al.*, 2018). Colour density, total anthocyanin content, total phenolics and methyl cellulose precipitable tannin as well as 27 individual phenolics were quantified.

Fermenting samples were collected in 2 mL eppies and centrifuged in an Eppendorf 5415D centrifuge (Hamburg, Germany) at 10 000 rpm for 5 minutes for clarification. One hundred µL of the supernatant was pipetted and diluted with 5 mL of 1 M Hydrochloric acid solution, capped and vortexed and placed in a dark cupboard followed by a waiting period of one hour. After the waiting period 200 µL of this mixture were pipetted into a UV-Visible Nunc F96 MicroWell plate (Nunc, Langenselbold, Germany) with 1M HCl as the reference blank. Samples were measured with the Multiskan GO Microplate Spectrophotometer (Thermo Fisher Scientific, Inc., Waltham, MA, USA) at 2 nm intervals over the wavelength range of 200 nm to 700 nm (Aleixandre-Tudo *et al.*, 2018).

## General analysis

The soluble solids (°Balling) of the grape juice were measured with a refractometer after crushing and destemming. The pH and total titratable acidity were measured with a 862 Compact Titrosampler instrument (Metrohm Ltd., Herisau, Switzerland). The finished wine was sampled before bottling and analysed with WineScan™ instrument (Foss Electric, Hillerød, Denmark). Free and total SO<sub>2</sub> were analysed with the Ripper method using the 702 SM Titrino instrument (Metrohm Ltd., Herisau, Switzerland) before bottling. Grape and wine data are reported in supplementary information.

### **3.2.5 STATISTICAL ANALYSIS**

#### Grape phenolic data

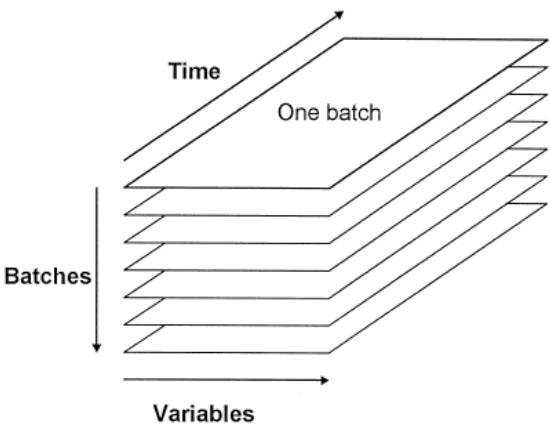
Analysis of variance (ANOVA) and post-hoc tests were conducted using Fishers LSD model  $p < 0.05$  to compare grape phenolic data. ANOVA and LSD post-hoc test was also used to evaluate the wines at the end of the fermentation process.

#### Fermenting data

BSPC (Batch statistical process control) is a multivariate statistical approach to process datasets generated during manufacturing processes providing an overview of batch development. The first phase entails batch evolution modelling (BEM) and provides an overview of the process progression. The second phase, so called batch level modelling (BLM) provides an overview of the overall batch behaviour during the entire process. The batch process data is thus condensed into a single data point in the scores space. This allows for a between batches comparison (i.e. location of the different data points in the scores space that correspond to the different treatments and batches) (Eriksson *et al.*, 2013). In our study a data set was generated for each grape batch (eight batches), with samples collected twice a day from twelve vinifications representing the three maceration times at two different levels of punch down over the alcoholic fermentation from crushing until end of fermentation. The completed data set consisted of 1920 fermenting wine samples. Both levels of BSPC were investigated in this study although BLM data is only reported.

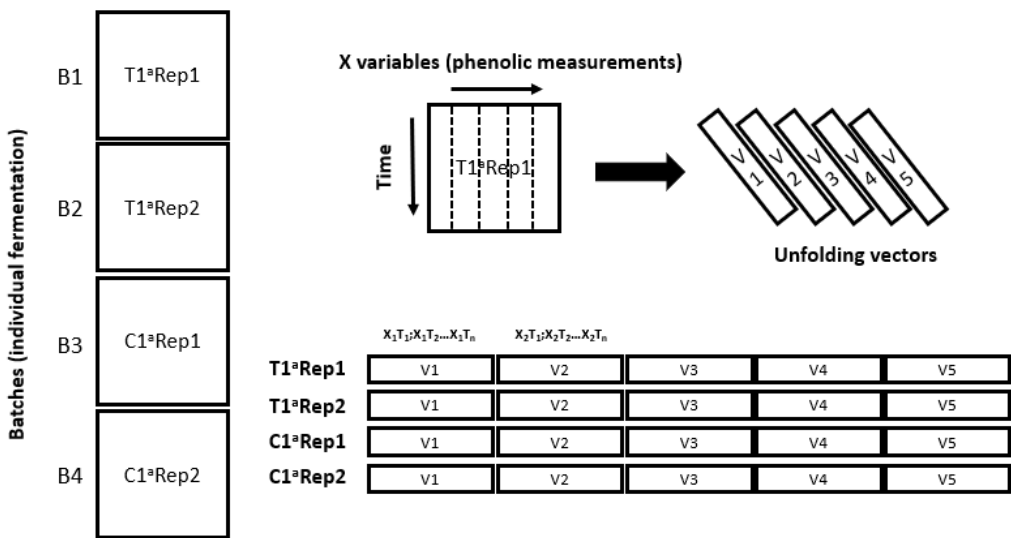
#### Multivariate modelling

The phenolic levels of the samples collected from initialization (crush & destem) until process completion (end of alcoholic fermentation) led to a three-way matrix for each grape batch (batch x observation x time). SIMCA 14.1 (Sartorius Stedim Biotech, Gotinga, Germany) was used for BSPC data analysis. Each grape batch is a representation of 12 treatment batches as observed in the experimental design (**Figure 3.2.2** and **Figure 3.2.3**).



**Figure 3.2.2:** A three-way matrix ( $N \times K \times J$ ) representing batch process data,  $J$  time points (maceration until end of alcoholic fermentation),  $K$  variables (individual phenolic compounds and parameters) and  $N$  treatment batches (12 wines) (Wold *et al.*, 1998).

Principal component analysis (PCA) and orthogonal partial least square discriminant analysis (OPLS-DA) models were developed during batch level modelling (BLM). As indicated, BLM includes all the batch information in a single row from time 0 until process completion as shown in **Figure 3.2.3**. The PCA score plot was plotted to explore differences between the batches. The scores plot shows each treatment batch as an overall observation in the score space. OPLS-DA modelling was used to evaluate if the different classes (treatments) can be discriminated and to investigate potential differences in the phenolic extraction within and between grape batches. The score values  $t$  represents a compression of all the variables measured during the fermentation process. The loadings plot provides information about the relationships among the variables (individual phenolic compounds) and provides a summary of phenolic extraction progression during the process. The loadings plots are complimentary to the score plot and it is used to better understand and interpret the phenolic extraction of the different treatments or batches. Loading plots showing every sampling point (from time 0 to process completion) are presented. The progression of the phenolic extraction during the fermentation can also be better visualized.

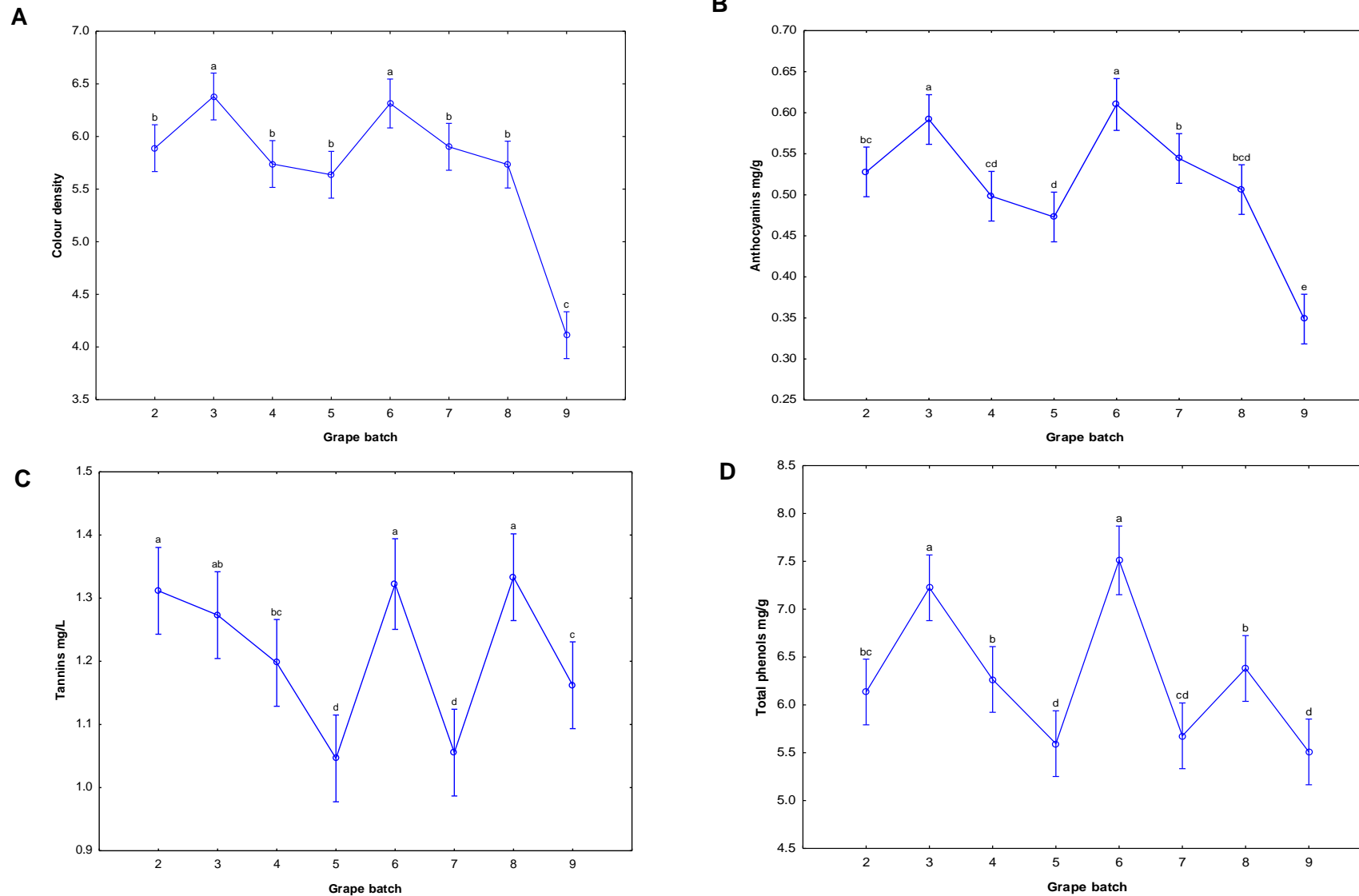


**Figure 3.2.3:** Batch data collected from initialisation until process completion, data transformed and represented in a single row.

### 3.3 RESULTS

#### 3.3.1 VARIABILITY OF GRAPE AND WINE PHENOLICS

The objective of the study was to source grapes from different vineyards in the Western Cape to introduce a certain degree of phenolic variability in our study. Two commercial grape varieties, Cabernet Sauvignon and Shiraz were selected for the study, as phenolic compositional differences by grape variety have been reported. Grape homogenate extracts were analysed to determine the phenolic composition within each grape batch. Significant differences were observed for the phenolic parameters' tannins, anthocyanins, colour density and total phenols between grape batches (**Figure 3.3.1**). However, in comparison with the other phenolic parameters, less significant differences were observed for colour density.



**Figure 3.3.1: Figure (A-D):** shows phenolic parameters measured namely colour density, total anthocyanins, total tannins and total phenols. B2, B6, B8 & B9-Cabernet Sauvignon grape batches. B3, B4, B5 & B7-Shiraz grape batches. Letters within graph indicate significant differences for Fischer's LSD at  $p < 0.05$

Since phenolic variability is an important objective in this study, soft independent modelling of class analogy (SIMCA) was used to validate our study by investigating phenolic content of fermenting samples during fermentation i.e. the entire data set collected during the course of fermentation was used in the SIMCA analysis. This method models each class separately. This cross-validation technique is applied to determine the critical distance i.e. border of each class model, determined by the residual standard deviation (DModX or distance to the model in x-space) of each training set (grape batch) used to predict a class for a new fermentation sample (Eriksson *et al.*, 2013). I.e. the residual distance of a new fermentation sample will be calculated and classified according to a class (batch) if the value is below the critical limit for that specific class (batch). For example, in **Figure 3.3.2.A** Cabernet Sauvignon fermentation samples 13-24 were correctly classified as fermentation samples from batch six, whereas fermentation samples 1-12 and 25-48, that correspond to the other three batches for this cultivar, did not fit the model, as their DModX values exceeded the critical distance. Batch six was therefore used to predict class of the other batches. This process is repeated until all batches are used to predict the remaining classes (batches). The same results were observed for the totality of the fermentation samples, classified accordingly to grape batch for both cultivars (**Figure 3.3.2** Cabernet Sauvignon and **Figure 3.3.3** Shiraz). These results confirm what have been observed in **Figure 3.3.1** for the grapes' phenolic content.

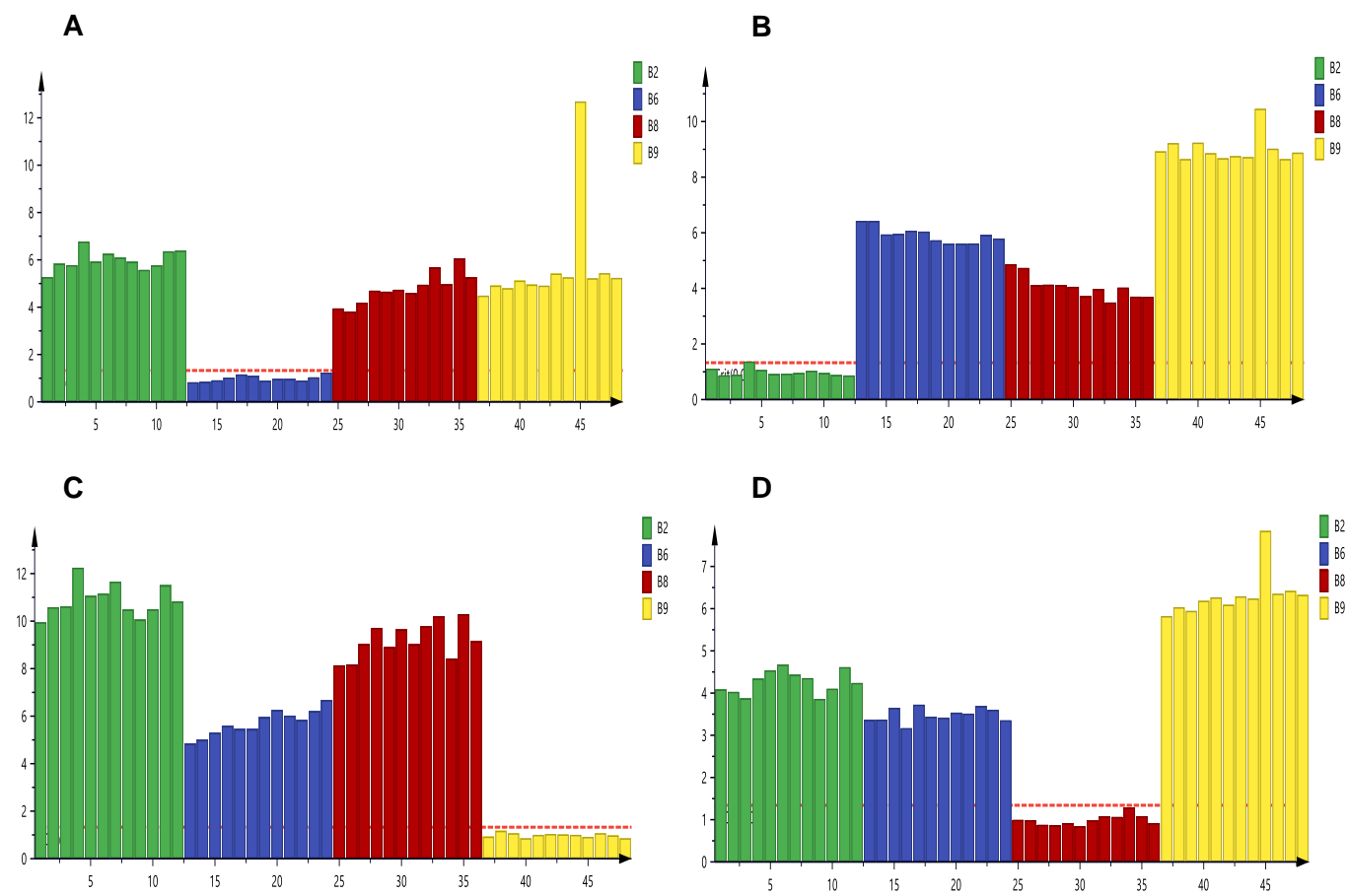
The first objective of the project was to ascertain if the effect of pressing time and cap management practices was constant regardless of grape and wine phenolic content and composition. In other words, if the results observed in one batch apply to other batches regardless of its grape and wine phenolic profile. It was therefore of importance to start off with grape batches with varying phenolic content and composition. This objective was confirmed in the ANOVA and SIMCA analysis results reported.

A batch level model (BLM) PCA plot was built to evaluate differences between wines made under different winemaking conditions. The BLM PCA score plot showed fermentation samples separating accordingly to the vineyard the grapes were sourced from. In the BLM PCA score plot (**Figure 3.3.4.A**) the fermentation samples separated in four groups according to the vineyard the Cabernet Sauvignon grapes were sourced from. Batch two (green) and eight (red), observed on the positive side (right side) of the PCA score plot, was associated with the majority of phenolic compounds that showed positive loading values (higher values of these measurements) (**Figure 3.3.4.B**). On the other hand, batch six (blue) and nine (yellow) observed on the negative side (left side) of the BLM PCA score plot was associated with higher levels of p-coumaric acid for instance, representing negative loading values.

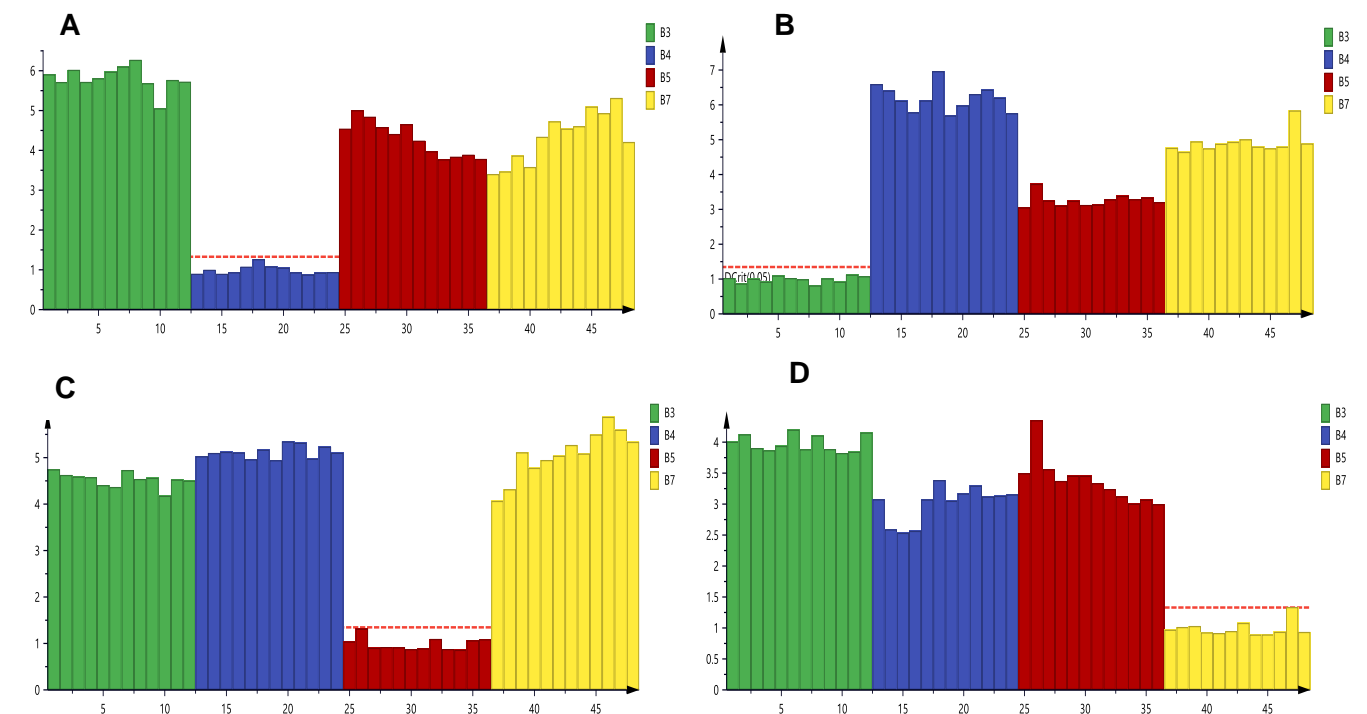
Similar results were observed in the BLM PCA score plot of Shiraz grape batches (**Figure 3.3.4.C**). The fermentation samples separated in four groups representing the vineyards the grapes were sourced from. In the PCA score plot batches three, five and seven were observed on the negative side (left) of the PCA score plot and were associated with the majority of phenolics that showed negative loading values. For example, these batches showed higher levels of anthocyanin content, caffeic acid, catechin, colour density, coumaric



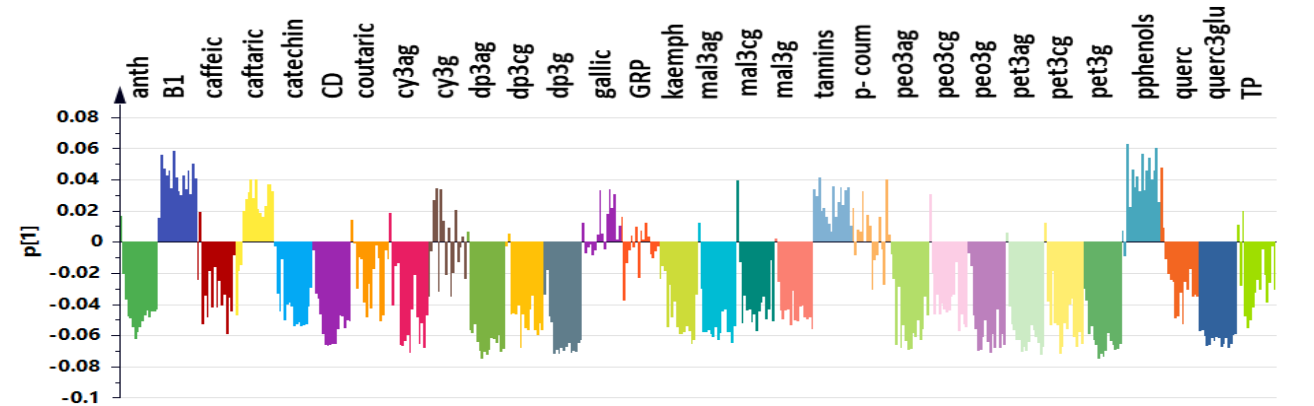
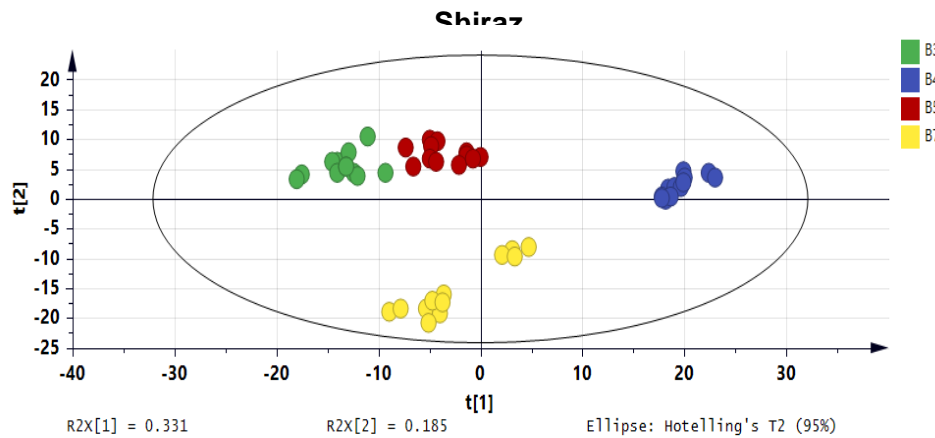
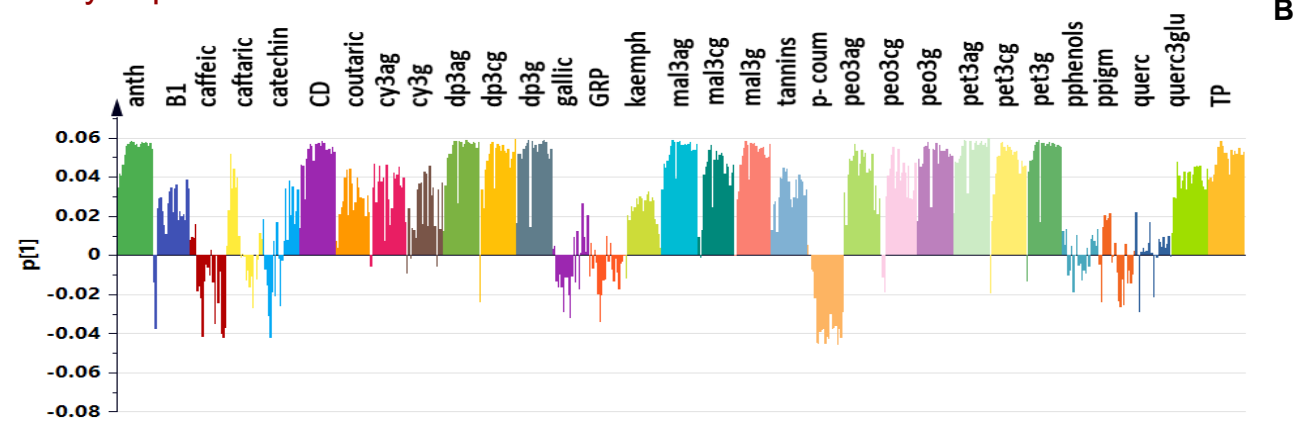
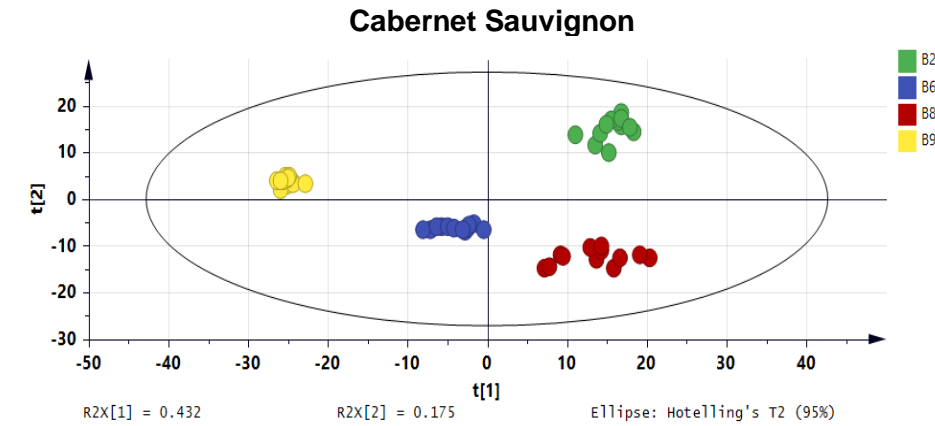
cid etc. whereas batch four observed on the positive side of the PCA score plot showed higher levels of B1 (dimer) content as well as polymeric phenol content (positive loading values) (**Figure 3.3.4.D**).



**Figure 3.3.2 (A-D):** DModX plot for the batch prediction set of the PCA model between Cabernet Sauvignon fermentation samples. The critical distance level is displayed by a red dotted line in each graph. The critical distance level value varies dependent of the data set. B2-green; B6-blue; B8-red; B9-yellow.



**Figure 3.3.3 (A-D):** DModX plot for the batch prediction set of the PCA model between Shiraz fermentation samples. The critical distance level is displayed by a red dotted line in each graph. The critical distance level value varies dependent of the data set. B3-green; B4-blue; B5-red; B7-yellow.



**Figure 3.3.4 (A-D):** Batch level modelling of Cabernet Sauvignon and Shiraz fermentation samples produced under different maceration conditions. **Figure A** and **C** shows PCA score plots ( $t_1$  vs  $t_2$ ) for Cabernet Sauvignon and Shiraz fermentation samples. **Figure B** and **C** shows the corresponding loadings plot  $p_1$  for the different models evaluated representing the individual phenolic compounds measured during alcoholic fermentation. B2, B6, B8 & B9-Cabernet Sauvignon grape batches. B3, B4, B5 & B7-Shiraz grape batches. Anth: total anthocyanins; B1: dimer B1; caffeic: caffeic acid; cat: catechin; CD: colour density; coutaric: coutaric acid; cy3ag: cyanidin-3-acetylglucoside; cy3g: cyanidin-3-glucoside; dp3ag: delphinidin-3-acetylglucoside; dp3cg: delphinidin-3-cumarylglucoside; dp3g: delphinidin-3-glucoside; gallic: gallic acid; GRP: grape reaction product; kaemph: kaempferol; mal3ag: malvidin-3-acetylglucoside; mal3cg: malvidin-3-cumarylglucoside; mal3g: malvidin-3-glucoside; tannins: total tannin content; *p*-coum: *p*-coumaric acid; peo3ag: peonidin-3-acetylglucoside; peo3cg: peonidin-3-cumarylglucoside; peo3g: peonidin-3-glucoside; pphenols: polymeric phenols; ppigm: polymeric pigments; querc: quercetin; querc3glu: quercetin-3-glucoside; TP: total phenols.

### 3.3.2 THE EFFECT OF PRESSING TIME (ABSENCE/PRESENCE OF SKINS) ON PHENOLIC EXTRACTION DURING ALCOHOLIC FERMENTATION

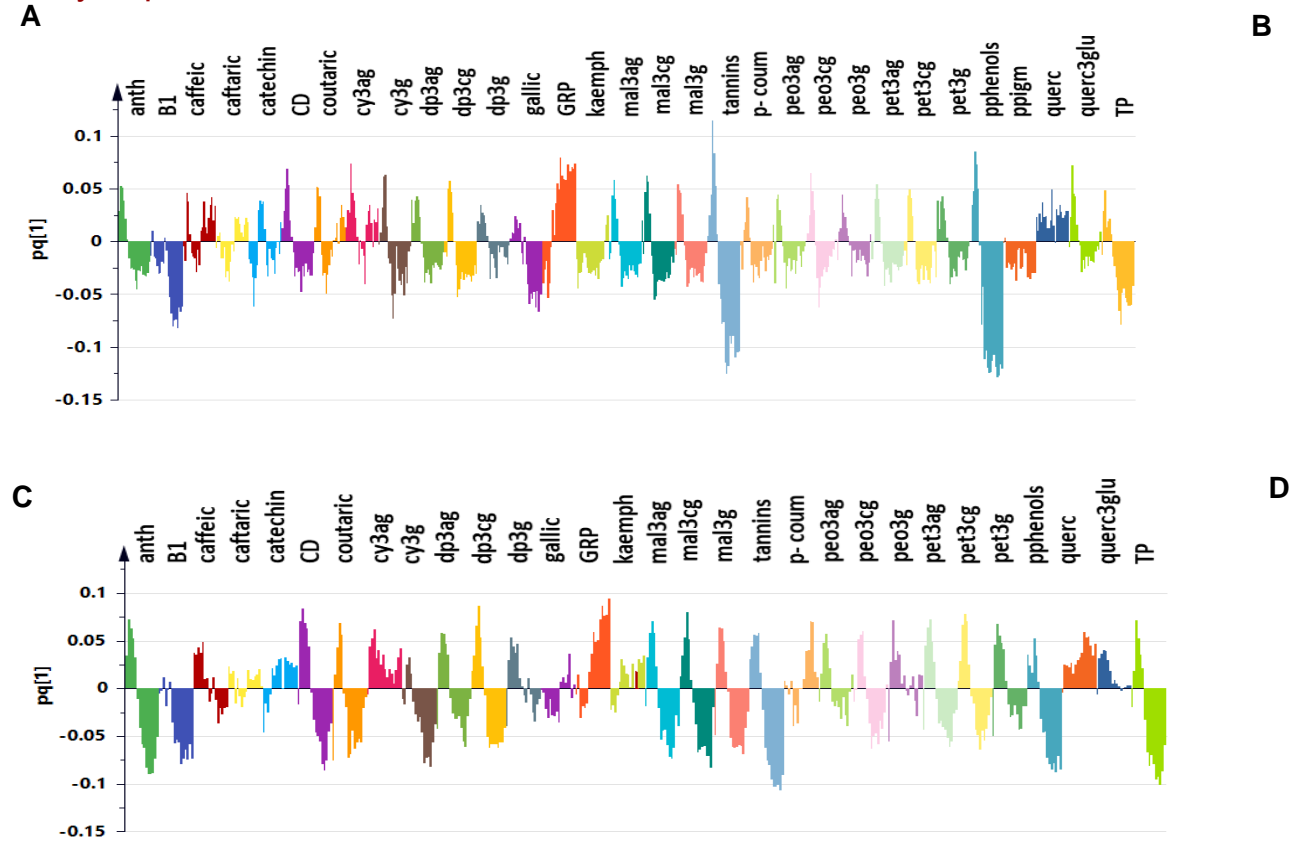
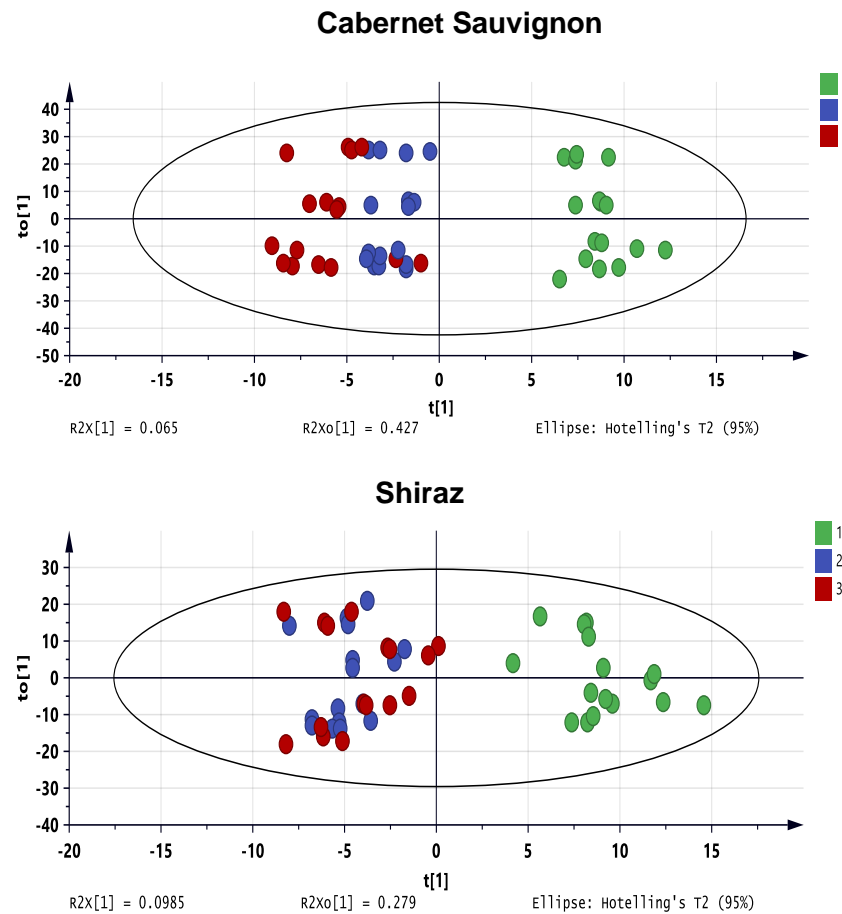
During alcoholic fermentation three different pressing times were investigated at two punch down levels (standard vs. increased punch down frequency). Regardless of the punch down frequency ( $T^a$  (increased) vs.  $C^b$  (standard)), the fermentation samples separated according to the pressing time. Therefore, the data represented in **section 3.3.2** is a combination of both punch down levels ( $T^a$  and  $C^b$ ) representing one of the three pressing times. The analysis per punch down frequency is however included in supplementary information **Figure S1** and **2**.

In the BLM OPLS-DA score plot (**Figure 3.3.5.A**) Cabernet Sauvignon fermentations separated in three groups representing the three different pressing times. Pressing time 1 to the right side of the OPLS-DA score plot displayed a good separation from pressing time 2 and 3 to the left side of the OPLS-DA score plot. A poor separation between pressing times 2 and 3 were displayed in the scatter plot. Regardless of grape variety, similar trends were observed for vinifications produced from Shiraz grapes. The fermentation samples separated accordingly to the three different pressing times, however overlapping of fermentation samples were observed to the left side of the OPLS-DA score plot representing pressing times 2 and 3 (**Figure 3.3.5.C**). The corresponding loadings plot (**Figure 3.3.5.B** and **D**) of the different OPLS-DA models provided information about phenolic extraction kinetics during alcoholic fermentation as they show loading values from the initial starting point (day 0) to the completion of fermentation. The loadings plot also revealed differences in phenolic content between the three pressing times. For example, a high tannin content (coloured light blue) in **Figure B** and **D** was at first associated with pressing time 1 with positive loading values for the first days of the fermentation, indicating higher values in those wines located in the positive part of the scores plot (pressing time 1 wines). However, as alcoholic fermentation progressed higher content of tannin was associated with pressing times 2 and 3 (negative loading values correlating with higher levels in the wines located in the negative side of the scores plot (pressing time 2 and 3). This concludes that initial extraction of phenolic compounds was associated with pressing time 1 followed by mid to end extraction corresponding with pressing times 2 and 3. Overall pressing times 2 and 3 were associated with higher phenolic content.

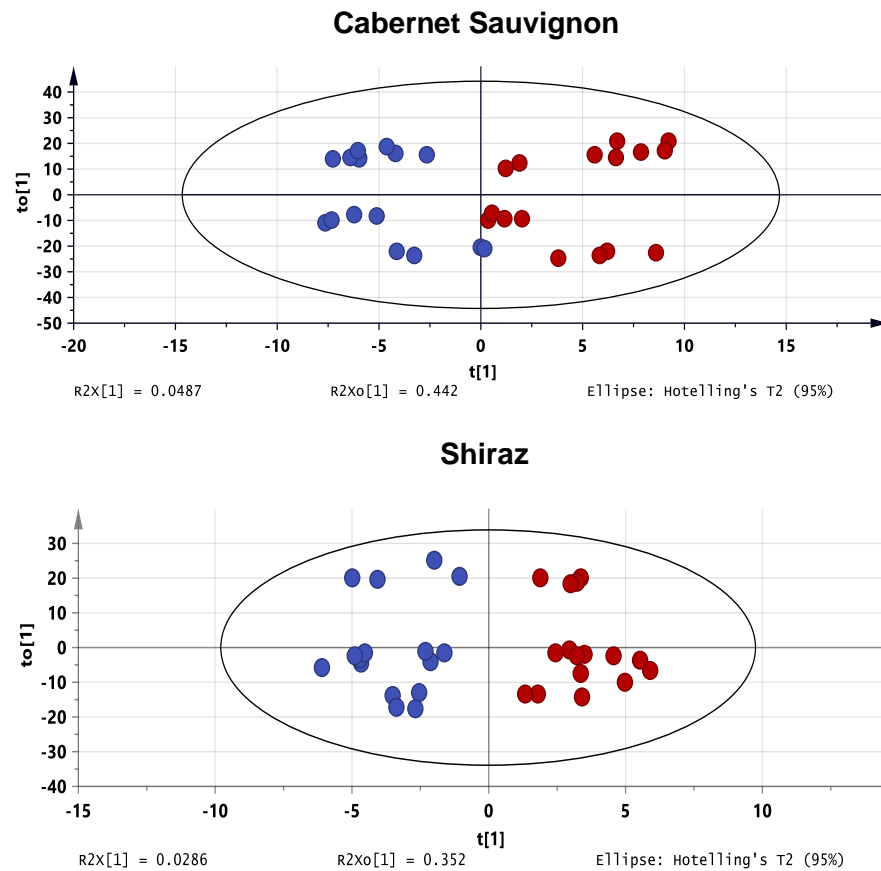
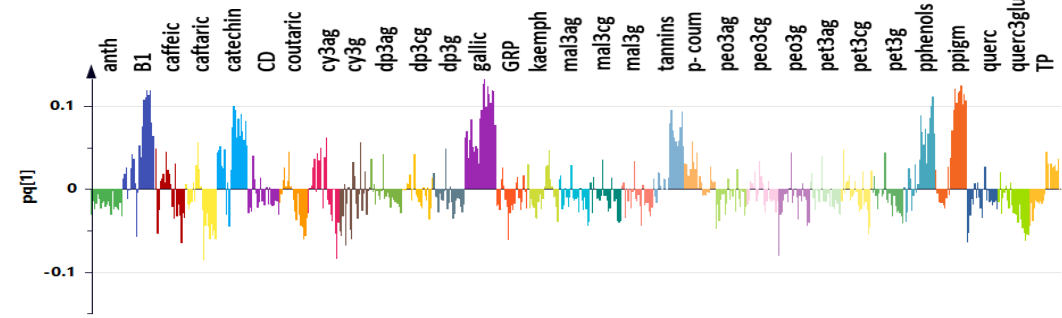
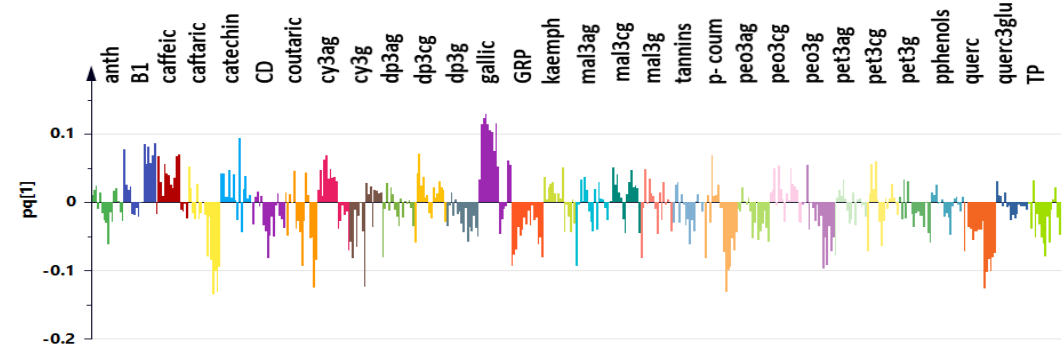
To further evaluate pressing times 2 and 3, an additional OPLS-DA models were built to investigate possible phenolic differences between pressing times. The OPLS-DA score plot showed good separation of Cabernet Sauvignon fermentation samples (**Figure 3.3.6 A**) in two groups representing pressing times 2 and 3. Pressing time 2 to the left side of the OPLS-DA score plot was well separated from pressing time 3 to the right side of the OPLS-DA score plot. Various phenolic compounds were responsible for the separation as indicated by the corresponding loadings plot. Pressing time 3 to the right side of the OPLS-DA score plot (positive side) were associated among others with, high levels of dimer B1, catechin, gallic acid, tannins, polymeric phenols and polymeric pigments (positive loadings).

However, pressing time 2 to the left side of the OPLS-DA score plot (negative side) were associated with higher levels of anthocyanin content (negative loadings).

Interestingly Shiraz fermentation samples separated according to pressing times 2 and 3 in the OPLS-DA score plot, however the corresponding loadings plot revealed a more similar phenolic content between the two pressing times (**Figure 3.3.6.D**). In general pressing time 3 showed higher levels of high gallic acid, catechin and dimer B1 content, among others. However, no clear effect was seen for polymeric phenols and tannins between pressing time 2 and 3. Interestingly pressing time 2 seems to be associated with higher levels of colour density and total phenol content.



**Figure 3.3.5 (A-D):** OPLS-DA score plots discriminating between the three pressing times for Cabernet Sauvignon and Shiraz fermentation samples. **Figure A** and **C** shows the OPLS-DA score plots ( $t_1$  vs  $to_1$ ) for Cabernet Sauvignon and Shiraz fermentation samples pressed at different stages of alcoholic fermentation. **Figure B** and **D** shows the corresponding loadings plot ( $p_1$ ) to the OPLS-DA models, coloured according to the measured variables. Each loading observed represents a time point (0-12 days) of the specific phenolic compound/parameter measured. 1-pressing time 1; 2-pressing time 2; 3-pressing time 3. Anth: total anthocyanins; B1: dimer B1; caffeic: caffeic acid; cat: catechin; CD: colour density; coutaric: coutaric acid; cy3ag: cyanidin-3-acetylglucoside; cy3g: cyanidin-3-glucoside; dp3ag: delphinidin-3-acetylglucoside; dp3cg: delphinidin-3-cumarylglucoside; dp3g: delphinidin-3-glucoside; gallic: gallic acid; GRP: grape reaction product; kaemph: kaempferol; mal3ag: malvidin-3-acetylglucoside; mal3cg: malvidin-3-cumarylglucoside; mal3g: malvidin-3-glucoside; tannins: total tannin content; *p*-coum: *p*-coumaric acid; peo3ag: peonidin-3-acetylglucoside; peo3cg: peonidin-3-cumarylglucoside; peo3g: peonidin-3-glucoside; pphenols: polymeric phenols; ppigm: polymeric pigments; querc: quercetin; querc3glu: quercetin-3-glucoside; TP: total phenols.


**A**

**B**
**C**

**D**

**Figure 3.3.6 (A-D):** OPLS-DA score plots discriminating between pressing times two and three for Cabernet Sauvignon and Shiraz fermentation samples. **Figure A** and **C** shows the OPLS-DA score plots (t1 vs to1) for Cabernet Sauvignon and Shiraz fermentation samples pressed at times 2 and 3. **Figure B** and **D** shows the corresponding loadings plot to the OPLS-DA models between pressing times 2 and 3. The loadings are coloured according to phenolic compounds measured during alcoholic fermentation. Each loading observed represents a time point (0-12 days) of the specific phenolic compound/parameter measured. 2-pressing time 2; 3-pressing time 3. Anth: total anthocyanins; B1: dimer B1; caffeic: caffeic acid; cat: catechin; CD: colour density; coutaric: coutaric acid; cy3ag: cyanidin-3-acetylglucoside; cy3g: cyanidin-3-glucoside; dp3ag: delphinidin-3-acetylglucoside; dp3cg: delphinidin-3-cumarylglucoside; dp3g: delphinidin-3-glucoside; gallic: gallic acid; GRP: grape reaction product; kaemph: kaempherol; mal3ag: malvidin-3-acetylglucoside; mal3cg: malvidin-3-cumarylglucoside; mal3g: malvidin-3-glucoside; tannins: total tannin content; *p*-coum: *p*-coumaric acid; peo3ag: peonidin-3-acetylglucoside; peo3cg: peonidin-3-cumarylglucoside; peo3g: peonidin-3-glucoside; pphenols: polymeric phenols; ppigm: polymeric pigments; querc: quercetin; querc3glu: quercetin-3-glucoside; TP: total phenols.

### 3.3.3 THE EFFECT OF PUNCH DOWN FREQUENCY APPLIED DURING MACERATION

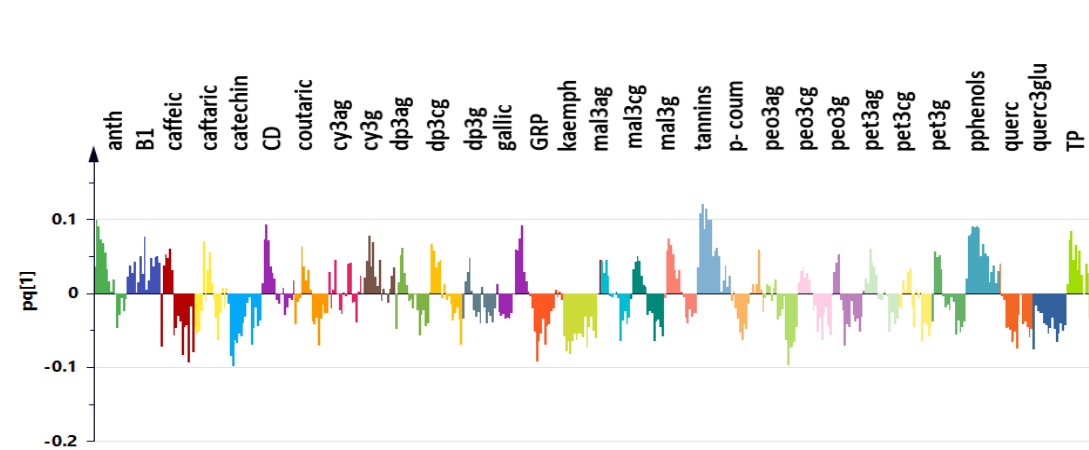
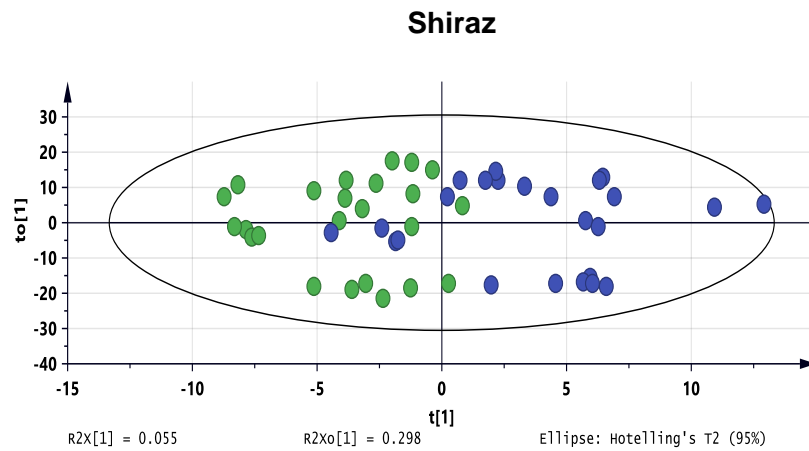
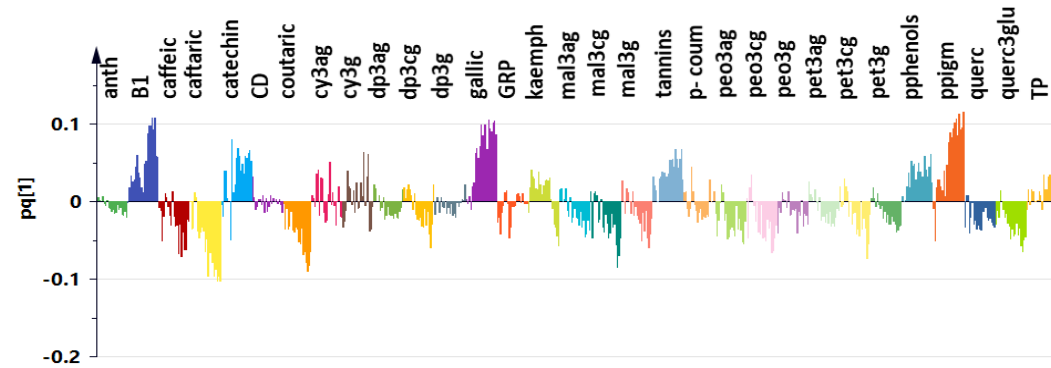
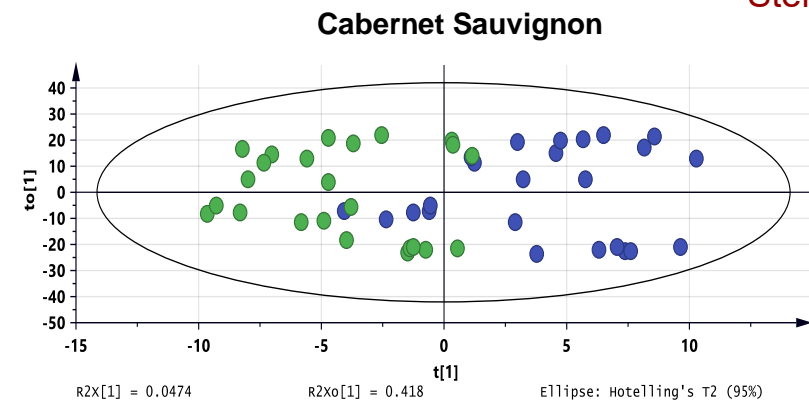
Punching down is a traditional method used in the wine industry to enhance phenolic extraction and maintain sufficient contact between the skins, seeds and juice. During alcoholic fermentation two levels of punch down (i.e. standard vs. increased) was investigated at three different stages of maceration (pressing time 1, 2 and 3).

As seen in the OPLS-DA score plot (**Figure 3.3.7.A**) Cabernet Sauvignon fermentation samples separated into two groups representing standard ( $C^b$ ) and increased ( $T^a$ ) punch down frequency. However, this separation was not that clear as when the effect of pressing time was evaluated with some overlapping samples. Increased punch down frequency to the right side of the OPLS-DA score plot was associated with higher content of dimer B1, catechin, gallic acid, tannins, polymeric phenols and polymeric pigments in the corresponding loadings plot. However, standard punch down frequency was associated with, among others high anthocyanin and phenolic acid content. Slightly different results were obtained for Shiraz vinifications produced with increased punch down frequency (**Figure 3.3.7.C**). The corresponding loadings plot revealed vinifications produced with increased punch down frequency were associated with high phenolic content such as anthocyanins, dimer B1, tannins, polymeric phenols and total phenols. Interestingly, Shiraz vinifications produced with standard punch down frequency were associated with high catechin content.

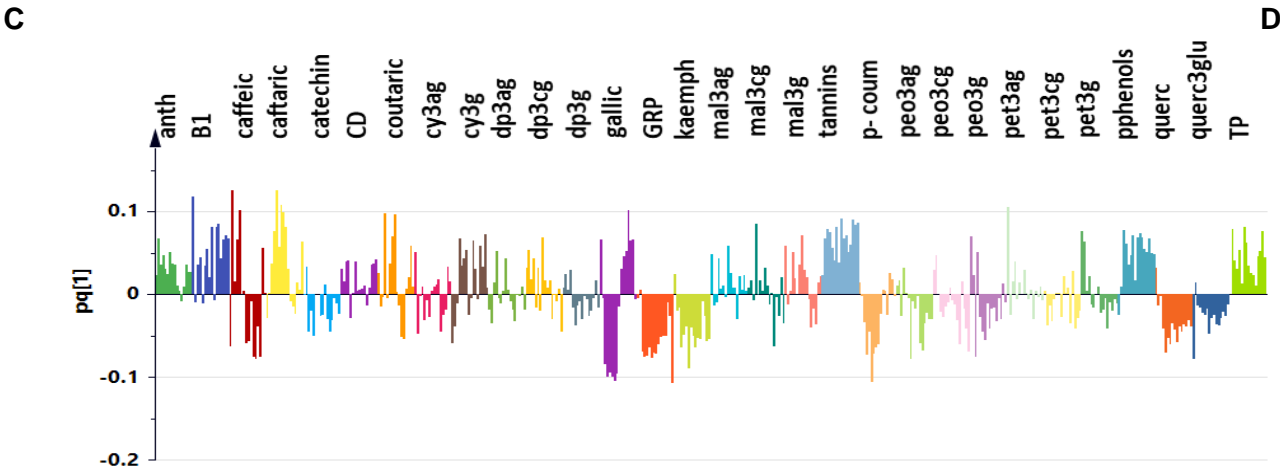
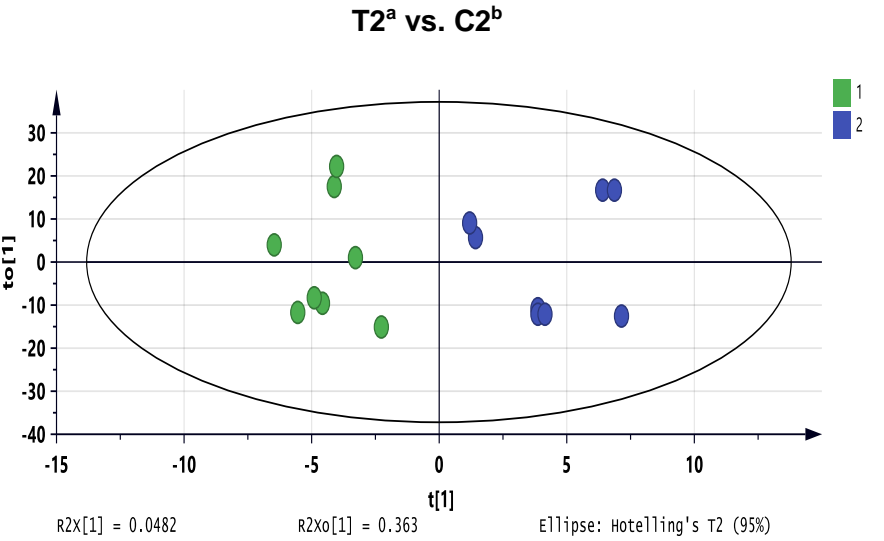
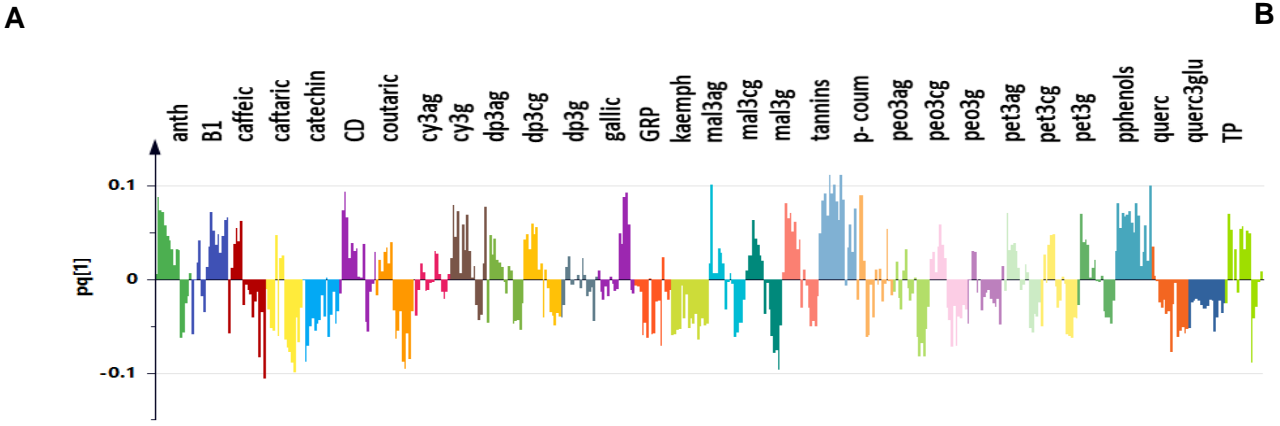
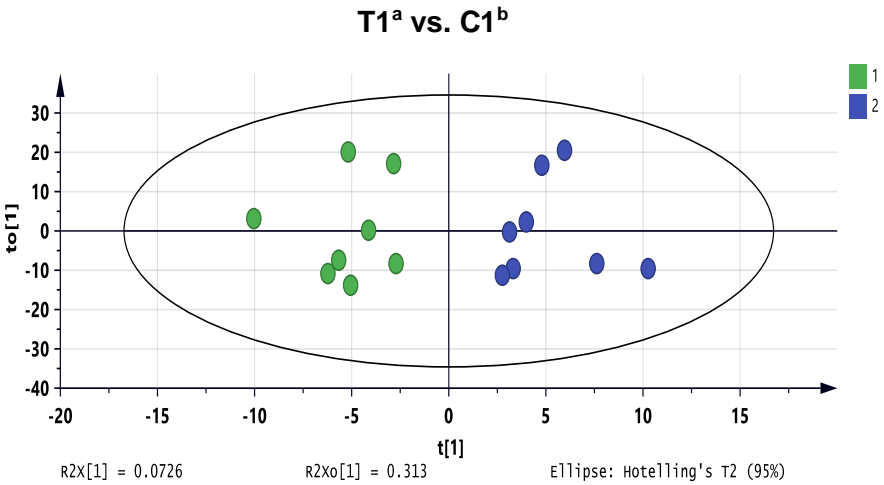
In addition, since overlapping and more scattered grouping was visible with all three pressing times combined, separate OPLS-DAs were created for each pressing time for both cultivars (i.e.  $C1^b$  vs  $T1^a$  etc.) to evaluate possible phenolic differences for the different pressing times or in other words to evaluate if the punch down effect was constant despite the pressing time. Overall similar results were observed for Cabernet Sauvignon as seen in the OPLS-DA score plots for the different pressing times (**Figure 3.3.8.A, C and E**).  $T^a$  was associated with high content of dimer B1, catechin, gallic acid, polymeric phenols and polymeric pigments for pressing time 1, 2 and 3, whereas  $C^b$  were associated with high anthocyanin content.

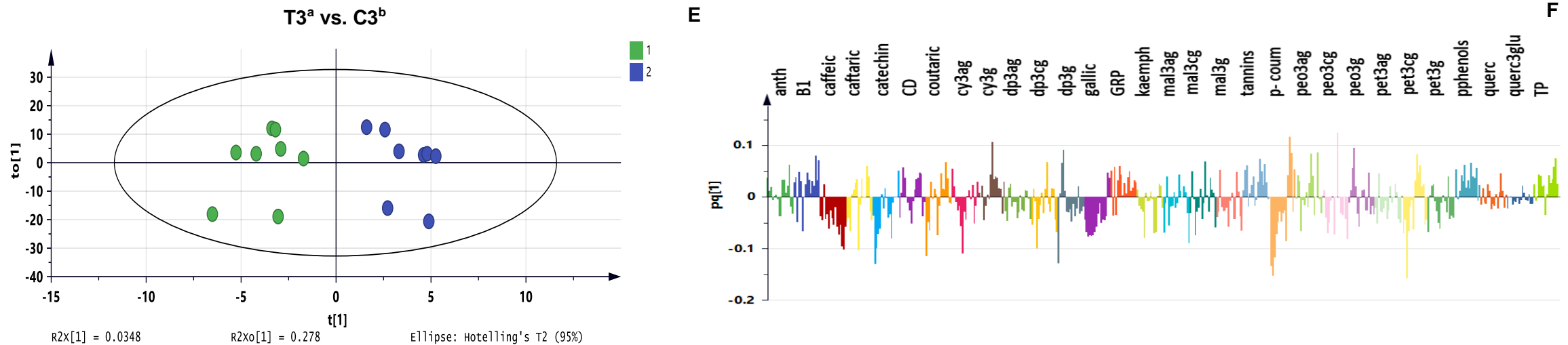
In general, similar results were observed within each pressing time for Shiraz fermentations.  $T^a$  was associated with high content of anthocyanins, dimer B1, tannins, polymeric phenols and total phenols. However,  $T3^a$  was not associated with high anthocyanin content. In addition,  $C1^b$  was associated with high phenolic acids, whereas control three was associated with high gallic acid content.



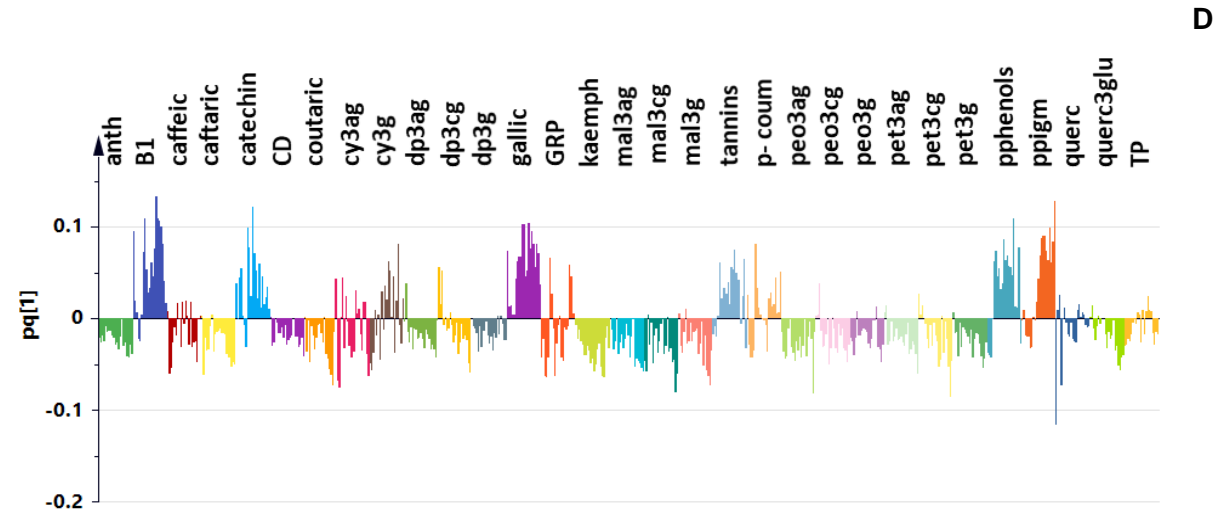
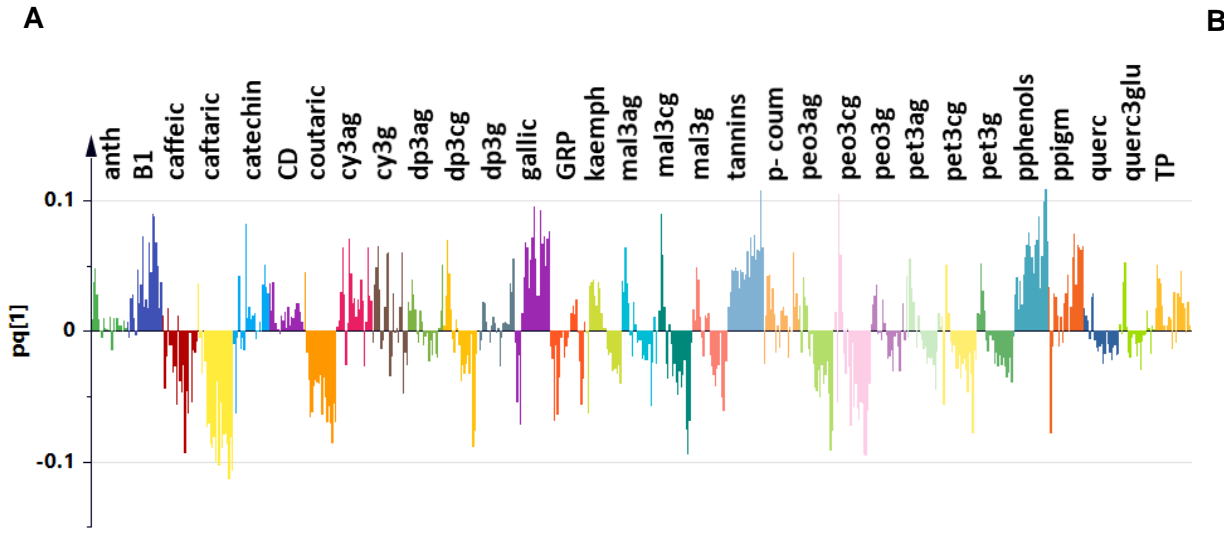
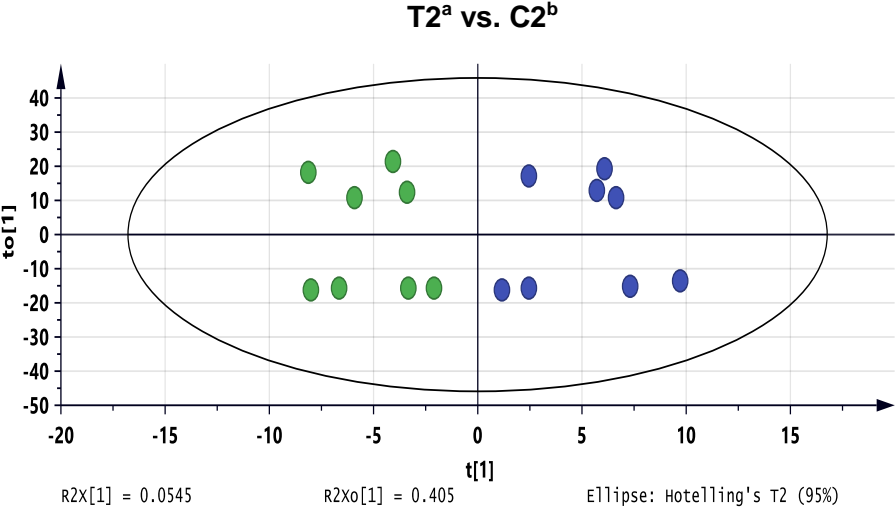
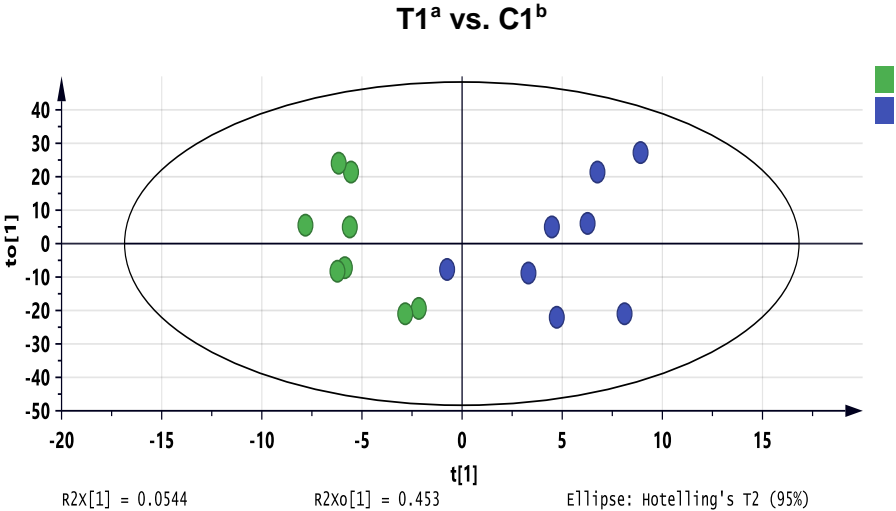


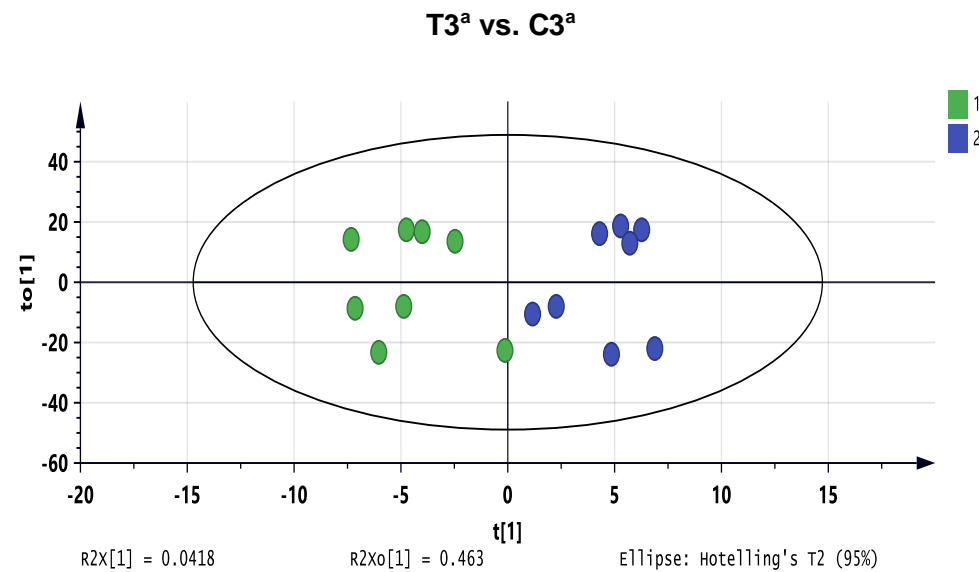
**Figure 3.3.7 (A-D):** OPLS-DA score plots discriminating between standard and increased punch down frequency applied during maceration. **Figure A** and **C** shows OPLS-DA score plots ( $t_1$  vs  $to_1$ ) of the overall punch down frequency effect of Cabernet Sauvignon and Shiraz fermentations produced under different maceration conditions. **Figure B** and **D** shows the corresponding loadings plot of the OPLS-DA models providing information about the phenolic variables contributing towards the separation. Each loading observed represents a time point (0-12 days) of the specific phenolic compound/parameter measured. 1-standard punch down frequency ( $C^b$ ); 2-increased punch down frequency ( $T^a$ ). Anth: total anthocyanins; B1: dimer B1; caffeic: caffeic acid; cat: catechin; CD: colour density; coutaric: coutaric acid; cy3ag: cyanidin-3-acetylglucoside; cy3g: cyanidin-3-glucoside; dp3ag: delphinidin-3-acetylglucoside; dp3cg: delphinidin-3-cumarylglucoside; dp3g: delphinidin-3-glucoside; gallic: gallic acid; GRP: grape reaction product; kaemph: kaempherol; mal3ag: malvidin-3-acetylglucoside; mal3cg: malvidin-3-cumarylglucoside; mal3g: malvidin-3-glucoside; tannins: total tannin content; *p*-coum: *p*-coumaric acid; peo3ag: peonidin-3-acetylglucoside; peo3cg: peonidin-3-cumarylglucoside; peo3g: peonidin-3-glucoside; pphenols: polymeric phenols; ppigm: polymeric pigments; querc: quercetin; querc3glu: quercetin-3-glucoside; TP: total phenols.



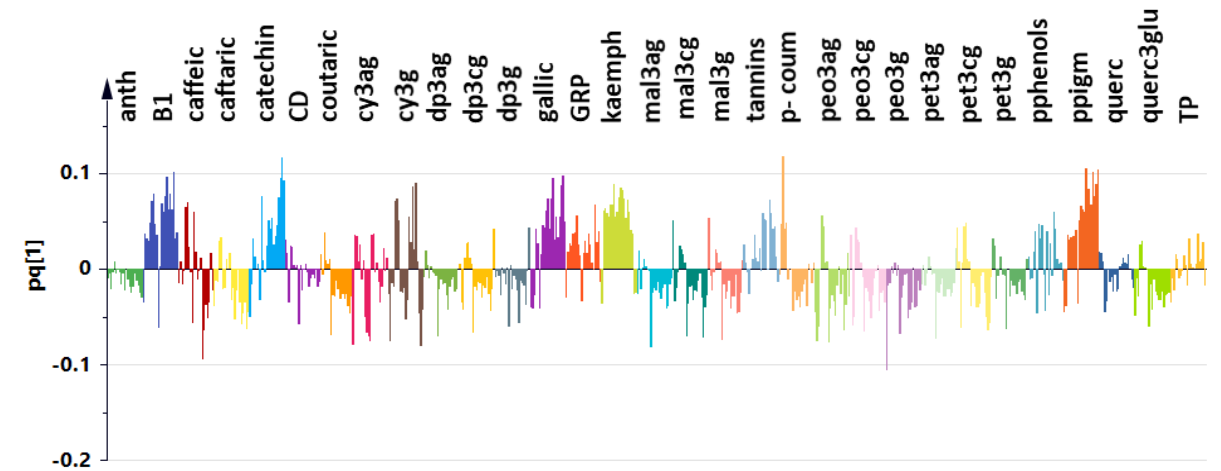


**Figure 3.3.8 (A-F):** OPLS-DA score plots discriminating between standard and increased punch down frequency for Shiraz fermentations. **Figure A, C and E** shows OPLS-DA score plots ( $t_1$  vs  $t_0$ ) for standard and increased punch down frequency for each pressing time (1, 2 and 3). **Figure B, D and F** shows the corresponding loadings plot of the OPLS-DA models and provides information about the phenolic compounds contributing towards the separation. Loadings are coloured according to measured variables. Each loading observed represents a time point (0-12 days) of the specific phenolic compound/parameter measured. 1-standard punch down frequency (C<sup>b</sup>); 2-increased punch down frequency (T<sup>a</sup>). Anth: total anthocyanins; B1: dimer B1; caffeic: caffeic acid; cat: catechin; CD: colour density; coutaric: coutaric acid; cy3ag: cyanidin-3-acetylglucoside; cy3g: cyanidin-3-glucoside; dp3ag: delphinidin-3-acetylglucoside; dp3cg: delphinidin-3-cumarylglucoside; dp3g: delphinidin-3-glucoside; gallic: gallic acid; GRP: grape reaction product; kaemph: kaempherol; mal3ag: malvidin-3-acetylglucoside; mal3cg: malvidin-3-cumarylglucoside; mal3g: malvidin-3-glucoside; tannins: total tannin content; *p*-coum: *p*-coumaric acid; peo3ag: peonidin-3-acetylglucoside; peo3cg: peonidin-3-cumarylglucoside; peo3g: peonidin-3-glucoside; pphenols: polymeric phenols; ppigm: polymeric pigments; querc: quercetin; querc3glu: quercetin-3-glucoside; TP: total phenols.





**E**



**F**

**Figure 3.3.9 (A-F):** OPLS-DA score plots discriminating between standard and increased punch down frequency of Cabernet Sauvignon fermentations. **Figure A, C** and **E** shows OPLS-DA models (t1 vs to1) for standard and increased punch down frequency for each pressing time (1, 2 and 3). **Figure B, D** and **F** shows the corresponding loadings plot of the OPLS-DA models between standard and increased punch down frequency and provides information about the phenolic compounds contributing towards the separation. The loadings are coloured according to phenolic compounds measured during alcoholic fermentation. 1-standard punch down frequency (C<sup>b</sup>); 2-increased punch down frequency (T<sup>a</sup>). Anth: total anthocyanins; B1: dimer B1; caffeic: caffeic acid; cat: catechin; CD: colour density; coutaric: coutaric acid; cy3ag: cyanidin-3-acetylglucoside; cy3g: cyanidin-3-glucoside; dp3ag: delphinidin-3-acetylglucoside; dp3cg: delphinidin-3-cumarylglucoside; dp3g: delphinidin-3-glucoside; gallic: gallic acid; GRP: grape reaction product; kaemph: kaempherol; mal3ag: malvidin-3-acetylglucoside; mal3cg: malvidin-3-cumarylglucoside; mal3g: malvidin-3-glucoside; tannins: total tannin content; *p*-coum: *p*-coumaric acid; peo3ag: peonidin-3-acetylglucoside; peo3cg: peonidin-3-cumarylglucoside; peo3g: peonidin-3-glucoside; pphenols: polymeric phenols; ppigm: polymeric pigments; querc: quercetin; querc3glu: quercetin-3-glucoside; TP: total phenols

### 3.3.4 PHENOLIC LEVELS OF THE FINAL WINES

In addition, analysis of variance (ANOVA) was applied at the last sampling point of alcoholic fermentation. The statistical approach was applied to determine statistically significant differences between treatments as observed in the BSPC analysis during the course of alcoholic fermentation. A clear separation was observed between pressing time 1 and pressing times 2 and 3 for both cultivars. The phenolic measurements anthocyanins, colour density, cyanidin-3-acetylglucoside, delphinidin-3-acetylglucoside, delphinidin-3-cumarylglucoside, gallic acid, malvidin-3-acetylglucoside, malvidin-3-cumarylglucoside, malvidin-3-glucoside, tannins, peonidin-3-acetylglucoside, petunidin-3-acetylglucoside, petunidin-3-cumarylglucoside, polymeric pigments and total phenols were found significantly higher for pressing times 2 and 3 for Cabernet Sauvignon (supplementary information **Table S3 and S4**). This indicates that the anthocyanins (individual compounds as well as total content and colour density) and tannin and phenolic fractions were found at higher levels in pressing time 2 and 3. However, when differences between pressing time 2 and 3 were further investigated, only the parameters gallic acid and polymeric pigments were found to be significantly higher in pressing time 3. These results are in agreement with the OPLS-DA analysis where differences were also observed between pressing times 2 and 3, however only statistically significant differences were observed for a few phenolic parameters at the end of the alcoholic fermentation.

In addition, a similar trend was observed in Shiraz fermentations. The phenolic parameters measured during alcoholic fermentation: anthocyanins, dimer B1, colour density, GRP, malvidin-3-acetylglucoside, tannins, peonidin-3-acetylglucoside, polymeric phenols, quercetin-3-glucoside and total phenols showed significantly higher levels in vinifications pressed at times 2 and 3. This again points towards an increased phenolic content in later pressing times including both anthocyanin (less prominent) and tannin related analysis as well as other phenolic classes as it is the case for GRP or quercetin-3-glucoside. Moreover, the flavanol dimer B1 was the only compound that was found at significantly higher levels in pressing time 3 when directly compared with pressing time 2 fermentations. These results seem to support what have been observed in the OPLS-DA (**Figure 3.3.5**), with more intense overlapping observed for Shiraz fermentations pressed at times 2 and 3.

Furthermore, ANOVA was also performed to evaluate the punch down effect at the end of the alcoholic fermentation process (supplementary information **Table S3 and S4**). The results showed Cabernet Sauvignon vinifications with significantly higher levels of catechin, tannins, polymeric pigments, quercetin and quercetin-3-glucoside produced with increased punch down frequency. However, in the case of Shiraz, the only compound found significantly higher were cyanidin-3-acetylglucoside as well as the phenolic parameter colour density, indicating a more effective effect of increased punch down frequency in Cabernet Sauvignon vinifications. These results were however not clearly reflected in the multivariate batch statistical approach, which is probably due to the latter taking the phenolic analyses performed during the whole course of fermentation into account. A more positive trend of increased punch down frequency was also observed in the OPLS-DA for Shiraz wines over the course of the fermentation process.

### 3.4. DISCUSSION

#### 3.4.1 VINEYARD COMPOSITIONAL VARIATION IN GRAPE AND WINE SAMPLES

Grape phenolic content was determined using the homogenate extraction protocol and showed that Cabernet Sauvignon grapes had higher tannin content in comparison with Shiraz grapes (Iland, 2000). These results are in agreement of what have been reported by Bindon *et al.* (2014). Numerous Shiraz and Cabernet Sauvignon homogenates were analysed and compared by Bindon *et al.* (2014) and it was found that Cabernet Sauvignon grapes were often characterised rich in tannin content. With regards to anthocyanin, total phenol and colour density content, grape batches three (Shiraz) and six (Cabernet Sauvignon) had significantly higher content. It is well known that flavonoid accumulation and composition e.g. tannins, anthocyanins and flavonols are greatly influenced by environmental factors such as soil composition, light, water vine status and temperature (Mori *et al.*, 2005, 2007; Downey *et al.*, 2006). However, as no data was collected regarding temperature, rainfall or vineyard management practices of the vineyards the grapes were sourced from, it is not possible to determine a causative effect for the observed phenolic compositional differences seen between grape batches.

On the other hand, comparing the phenolic content of the corresponding fermentations, vineyard related phenolic differences were not consistent from grape to wine. Grapes with significantly high phenolic content did not necessarily produce wine rich in phenolic content. This may be due to numerous factors influencing extractability from the grape to the must (Garrido-Bañuelos *et al.*, 2019). Interestingly, both Cabernet Sauvignon (**Figure 3.3.3.A** and **B**) and Shiraz (**Figure 3.3.4.C** and **D**) fermentation samples separated in the batch level model (BLM) PCA score plot accordingly to the vineyards the grapes were sourced from (**Table 3.2.1**), however the phenolic content from grape to wine could have possibly been influenced by the maceration conditions as well. The vineyard effect on phenolic content observed in the BLM PCA score plot most likely reflects the interaction of vineyard management practices applied, climatic conditions and degree of ripeness the grapes were harvested at. Nevertheless, grape variety is another important factor that needs to be taken into consideration in terms of phenolic differences being observed in the BLM PCA score plot. General high phenolic content was associated with fermentation batches two, three, five, seven and eight, however initially grape batches three and six had significantly higher phenolic content as seen in **Figure 3.3.1.** and **Figure 3.3.4.**

#### 3.4.2 THE INFLUENCE OF SKIN CONTACT TIME ON PHENOLIC CONTENT

The influence of different winemaking practices has been investigated in several studies to improve overall wine quality by enhancing phenolic extraction during maceration (Smith *et al.*, 2015). As highlighted in the review of Sacchi *et al.* (2005) various practices and variables can have an influence on the phenolic content of red wine. Phenolic content can be enhanced or modified with different winemaking techniques such as cold maceration,



thermovinification, extended maceration and must freezing. However, various studies have reported the management of skin contact time as the most crucial factor influencing phenolic content and sensory attributes (Casassa and Harbertson, 2014).

Numerous fermentation samples (1920) were collected during the maceration process of two commercial cultivars and 31 individual phenolic compounds were measured. In general, Cabernet Sauvignon and Shiraz grape batches pressed at 2/3<sup>rd</sup>s and near the end of alcoholic fermentation were associated with higher phenolic content, whereas fermentations pressed after two days of maceration was associated with low phenolic content. These results are in agreement with numerous studies regarding phenolic extraction kinetics during maceration and validates the effectiveness of batch level modelling as a rapid analytical method to measure and monitor phenolic extraction during fermentation, but as well as potentially predictive tool to modify the phenolic profile in future red wine productions (Romero-Cascales *et al.*, 2005; Bautista-Ortín *et al.*, 2016).

Longer maceration conditions often resulted in red wines with especially higher tannin and polymeric phenol content. This is in agreement with other studies concluding longer maceration conditions resulted in wines with increased tannin and polymeric phenol formation (Gómez-Plaza *et al.*, 2001; Romero-Cascales *et al.*, 2005; Sacchi *et al.*, 2005; Casassa *et al.*, 2013; Casassa and Harbertson, 2014). Literature has highlighted skin and seed tannins follow different extraction kinetics (Casassa *et al.*, 2013). Skin tannins are extracted during the early stages of fermentation and will reach a plateau, whereas seed tannin will increase linearly if maceration is extended (Cerpa-Calderón and Kennedy, 2008). Pressing at different stages of maceration possibly modified the tannin composition and content of fermentations pressed at times 2 and 3. In addition, other factors such as temperature and alcohol can also favour the extraction of phenolic compounds (Zimman *et al.*, 2002; Hernández-Jiménez *et al.*, 2012). It is well known that during the process of alcoholic fermentation, alcohol content increases as the fermentation progresses. In addition, increased alcohol content is especially favourable for seed tannin extraction and may contribute towards increased phenolic content (Hernández-Jiménez *et al.*, 2012).

On the other hand, fermentations pressed at 1/3<sup>rd</sup> of alcoholic fermentation often contained lower phenolic content. Optimum phenolic extraction entails sufficient skin contact time between the skins and the juice, since the desired phenolic compounds such as anthocyanins and tannins are located in the berry skins. Romero-Cascales *et al.* (2005) reported anthocyanin extraction reaches a maximum at day seven of maceration, whereas fermentations pressed at 1/3<sup>rd</sup> of alcoholic fermentation in our experiments were only in contact with the skins for two days. Lower anthocyanin concentrations would be expected from fermentations pressed at time 1. In addition, anthocyanin and tannin extraction follows similar kinetics regardless of grape variety (Bautista-Ortín *et al.*, 2016). Similar trends were observed for Shiraz grape batches evaluating the effect of skin contact time on phenolic content during different stages of maceration (**Figure 3.3.5.C and D**). The fermentation samples separated in the BLM OPLS-DA score plot accordingly to the three different pressing times 1, 2 and 3. Fermentations pressed at 1/3<sup>rd</sup> of alcoholic fermentation (pressing time 1) was associated with lower phenolic content, whereas fermentations pressed at 2/3<sup>rd</sup>s

and near the end of alcoholic fermentation (pressing time 2 and 3) were associated with higher polymeric phenolic and tannin content.

Additionally, taking a closer look at the Cabernet Sauvignon fermentation samples pressed at times 2 and 3 phenolic differences between pressing time 2 and 3 were also observed (**Figure 3.3.6.A and B**). In general fermentations pressed at 2/3<sup>rd</sup>s of alcoholic fermentation contained higher anthocyanin content compared to fermentations pressed near the end of alcoholic fermentation. These results are in agreement with our current knowledge regarding anthocyanin kinetics during alcoholic fermentation (Bautista-Ortín *et al.*, 2016). Anthocyanins are extracted in the first few days of maceration, however anthocyanin content can start to decrease with longer maceration times due to yeast cell reabsorption, degradation, refixation on the skins or due to polymeric pigment formation (González-Neves *et al.*, 2012). Pressing at 2/3<sup>rd</sup>s of alcoholic fermentation were probably more favourable conditions for high anthocyanin content compared to pressing near the end of alcoholic fermentation. With regards to tannin content, fermentations pressed near the end of alcoholic fermentation were associated with higher levels. Higher tannin concentrations were to be expected, since proanthocyanidin content can be modified by managing the maceration length (Casassa and Harbertson, 2014). Ivanova *et al.* (2012) concluded seed proanthocyanidin extraction were driven by maceration length and alcohol content. Increased tannin and polymeric phenol content were probably due to increased seed tannin extraction with longer skin contact time. Authors have reported seed tannins contributes towards the majority of total wine tannins with longer maceration conditions (Harbertson *et al.*, 2009). In addition, longer maceration conditions promote hydration of the grape seeds and may cause increased gallic acid extraction from seeds as well (Lerno *et al.*, 2015). This was observed comparing fermentation samples of pressing times 2 and 3, where the latter contained higher levels of gallic acid.

Fewer phenolic differences were observed investigating the Shiraz fermentation samples pressed at time 2 and 3 (**Figure 3.3.6.C and D**). However, similar trends were obtained compared to Cabernet Sauvignon fermentations pressed near the end of alcoholic fermentation. Fermentations pressed at time 3 were associated with higher gallic acid content, whereas no clear difference in terms of tannin content could be observed. Longer maceration conditions were probably favourable for seed hydration and could have possibly increased gallic acid extraction near the end of alcoholic fermentation (Cerpa-Calderón and Kennedy, 2008). However, with regards to tannin differences observed between Cabernet Sauvignon and Shiraz vinifications, tannin composition is greatly influenced by grape variety. Busse-Valverde *et al.* (2010) reported the proanthocyanidin profiles of Cabernet Sauvignon and Shiraz wines were more dependent on grape variety itself than the winemaking practices applied. It is well known that certain cultivars are richer in phenolic content compared to others (du Toit and Visagie, 2012). Cabernet Sauvignon have been characterized as a cultivar high in tannin content, whereas Shiraz is known to be high in anthocyanin content. However, tannin structure and concentration may be variety dependent, extraction may be also influenced by cell wall composition (Mattivi *et al.*, 2009). In addition of the cell wall composition, the degree of ripeness the grapes are harvested at may also influence the ease of extractability from grape to must (Harbertson *et al.*, 2009).

Grapes harvested at riper levels have been reported to increase extractability of phenolics such as anthocyanins and tannins (Canals *et al.*, 2005).

### 3.4.3 THE EFFECT OF THE PUNCH DOWN FREQUENCY APPLIED DURING MACERATION

Cap management is an important practice influencing the extraction of phenolic compounds during maceration. Optimum contact between the skins, seeds and the juice are essential for the diffusion process of desired phenolics. However, few studies have investigated the effect of cap management during fermentative maceration (Ichikawa *et al.*, 2012; Smith *et al.*, 2015; Lerno *et al.*, 2018). Phenolic differences were observed between the two punch down levels during maceration for both Cabernet Sauvignon and Shiraz fermentations (**Figure 3.3.7**). Increased punch down frequency was associated with higher levels of phenolic compounds such as dimer B1, catechin, gallic acid, tannins, polymeric phenols and polymeric pigments for Cabernet Sauvignon (**Figure 3.3.7.A and B**). Similar phenolic differences were observed for Shiraz, however high anthocyanin content was associated with increased punch down frequency for Shiraz fermentation samples (**Figure 3.3.7.C and D**). The ease of extractability from the grape to the must may have contributed to these results. It has been reported that anthocyanin extraction reaches an equilibrium by day six or seven of alcoholic fermentation, limiting further extraction. Bautista-Ortín *et al.* (2016) reported 80% of anthocyanins were extracted from Cabernet Sauvignon grapes at maximum extraction time point, however only 67% of anthocyanins were extracted from Shiraz grapes. Increased punch down frequencies could have led to mechanical disruption of the Shiraz skins, leaching anthocyanins and increasing content. Enhanced polymeric phenol, tannin and gallic acid content were to be expected, due to their localization in grape seeds. In addition, the authors Fischer *et al.* (2000) reported enhanced mechanical disruption increased seed tannin extraction.

With regards to standard punch down frequency, Cabernet Sauvignon fermentations were associated with among others, high anthocyanin and phenolic acid content. Lower anthocyanin content might occur with increased punch down frequency, since mechanical disruption of the grape tissue could have led to re-fixation on the skins or seeds (Ichikawa *et al.*, 2012). Loss of anthocyanin content could also be due to adsorption by yeast cells or participation in oxidation or condensation reactions (Bautista-Ortín *et al.*, 2016). In addition, high phenolic acid content can probably be expected with standard punch down frequencies, since these phenolic acids are most abundant in free-run juice (Teixera *et al.*, 2013).

These results contribute to other studies that have reported manual punch down as an effective method to enhance phenolic extraction during fermentative maceration. However, previous research have indicated varying results in terms of cap management practices applied (punch downs, pump overs etc.) indicating results were grape dependent, whereas these different mixing practices had small effect on phenolic extraction and final content (Sacchi *et al.*, 2005; Lerno *et al.*, 2018).

### 3.5 CONCLUSION

Strategic management of phenolic content is important, since red wine quality have been associated with higher anthocyanin and tannin content. This study showed the suitability of batch level modelling in combination with UV-Vis spectroscopy PLS calibration models to measure and monitor phenolic extraction during red wine production under different maceration conditions. Batch level modelling provided an overview of overall batch behaviour for each pressing time as well as between the three pressing times. In general, the fermentation samples separated accordingly to the three pressing times regardless of grape variety. Longer skin contact time proved to enhance polymeric phenol and tannin levels. However, Cabernet Sauvignon seems to be a more suitable cultivar for longer maceration conditions, since more clear trends were observed between phenolic extraction up until 2/3<sup>rd</sup>s of alcoholic fermentation compared to the influence of skin contact time until the end of alcoholic fermentation, whereas fewer phenolic differences were observed for Shiraz. Additionally, the absence/presence of skins seems to have modified the phenolic content of the final wines to a large extent, proving management of skin contact time as a crucial factor that can be used to modify phenolic content. Furthermore, batch level modelling displayed phenolic differences between standard and increased punch down regimes with similar trends observed for both cultivars. However, some differences were observed between Cabernet Sauvignon and Shiraz that may be cultivar dependent as reported by previous authors. Increased punch downs were often associated with the majority of phenolic compounds. Finally, the results showed in this study, validated the effectiveness of spectroscopy PLS calibration models to monitor phenolic extraction during maceration, proving to be a suitable, rapid and cost-effective method.

### 3.6 LITERATURE CITED

- Aleixandre-Tudo, J. L., Buica, A., Nieuwoudt, H. and du Toit, W. J. (2017). Spectrophotometric analysis of phenolic compounds in grapes and wines. *J. Agric. Food Chem.*, 65, 4009-4026.
- Aleixandre-Tudo, J. L., Nieuwoudt, H., Olivieri, A. and du Toit, W. J. (2018). Phenolic profiling of grapes, fermenting samples and wines using UV- Visible spectroscopy with chemometrics. *Food Control*, 85, 11-22.
- Aleixandre-Tudo, J. L. and du Toit, W. J. (2019). Understanding cold maceration in red winemaking: A batch processing and multi block data analysis approach. *Food Sci. Tech.*, 111, 147-157.
- Bautista-Ortín, A. B., Busse-Valverde, N., Fernández-Fernández, J. I., Gómez-Plaza, E. and Gil-Muñoz, R. (2016). The extraction kinetics of anthocyanins and proanthocyanidins from grape to wine. *J. Int. Sci. Vigne Vin*, 50, 91-100.
- De Beer, D., Joubert, E., Marais, J. and Manley, M. (2006). Maceration before and during fermentation: Effect on Pinotage wine phenolic composition, total antioxidant capacity and objective colour parameters. *S. Afric. J. Enol. Vitic.*, 27(2), 137-150.
- Bindon, K. A., Kassara, S., Cynkar, W. U., Robinson, E. M. C., Scrimgeour, N. and Smith, P. A. (2014). Comparison of extraction protocols to determine differences in wine-extractable tannin and anthocyanin in *Vitis vinifera* L. cv. Shiraz and Cabernet Sauvignon grapes. *J. Agric. Food Chem.*, 62(20), 558-4570.
- Bindon, K. A., Smith, P. A. and Kennedy, J. A. (2010) Interaction between grape-derived proanthocyanidins and cell wall material. 1. Effect on proanthocyanidin composition and molecular mass. *J. Agric. Food Chem.*, 58, 2520-2528.

- Busse-Valverde, N., Gómez-Plaza, E., López-Roca, J. M., Gil-Muñoz, R., Fernández-Fernández, J. I. and Bautista-Ortín, A. B. (2010). Effect of different enological practices on skin and seed proanthocyanidins in three varietal wines. *J. Agric. Food Chem.*, 58(21), 11333-11339.
- Canals, R., Llaudy, M. C., Vall, J., Canals, J. M. and Zamora, F. (2005). Influence of ethanol concentration on the extraction of color and phenolic compounds from the skin and seeds of tempranillo grapes at different stages of ripening. *J. Agric. Food Chem.*, 53, 4019-4025.
- Casassa, L. F., Beaver, C. W., Mireles, M. S., Larsen, R. C., Hopfer, H., Heymann, H. and Harbertson, J. F. (2013). Influence of fruit maturity, maceration length, and ethanol amount on chemical and sensory properties of Merlot wines. *Am. J. Enol. Vitic.*, 64(4), 437-449.
- Casassa, L. F. and Harbertson, J. F. (2014). Extraction, evolution, and sensory impact of phenolic compounds during red wine maceration. *Annu. Rev. Food Sci. Technol.*, 5, 83-109.
- Cerpa-Calderón, F. K. and Kennedy, J. A. (2008). Berry integrity and extraction of skin and seed proanthocyanidins during red wine fermentation. *J. Agric. Food Chem.*, 56, 9006-9014.
- Cheynier, V., Hidalgo Arellano, I., Souquet, J. M. and Moutounet, M. (1997). Estimation of the oxidative changes in phenolic compounds of Carignane during winemaking. *Am. J. Enol. Vitic.*, 48, 225-228.
- Cheynier, V., Salas, E., Souquet, J. M., Sarni-Manchado, P., Maury, C. and Fulcrand, H. (2006). Structure and properties of wine pigments and tannins. *Am. J. Enol. Vitic.*, 57(3), 298-305.
- Chittenden, R., Annand, M., King, P. and Russell, G. (2015). The effect of half plunging and no plunging as alternative winemaking techniques on phenolic extraction and pigment composition of wine. *S. Afr. J. Enol. Vitic.*, 36(1), 134-145.
- Cozzolino, D. (2015). The role of visible and infrared spectroscopy combined with chemometrics to measure phenolic compounds in grape and wine samples. *Molecules*, 20, 726-737.
- Downey, M. O., Dokoozlian, N. K. and Krstic, M. P. (2006). Cultural practice and environmental impacts on the flavonoid composition of grapes and wine: a review of recent research. *Am. J. Enol. Vitic.*, 57(3), 257-268.
- Eriksson, L., Byrne, T., Johansson, E., Trygg, J. and Vikstrom, C. (2013). *Multi- and Megavariate Data Analysis Basic Principles and Applications*. Umetrics Academy, 3<sup>rd</sup> edn, Umeå, Sweden.
- Casassa, L. F., Beaver, C. W., Mireles, M. S. and Harbertson, J. F. (2013). Effect of extended maceration and ethanol concentration on the extraction and evolution of phenolics, colour components and sensory attributes of Merlot wines. *Aus. J. Grape Wine Res.*, 19, 25-39.
- Fischer, U., Strasser, M. and Gutzler, K. (2000). Impact of fermentation technology on the phenolic and volatile composition of German red wines. *Int. J. Food Sci. Technol.*, 35, 81-94.
- García-Beneytez, E., Revilla, E. and Cabello, F. (2002). Anthocyanin pattern of several red grape cultivars and wines made from them. *Eur. Food Res. Technol.*, 215, 32-37.
- Garrido-Bañuelos, G., Buica, A., Schückel, J., Zietsman, A. J. J., Willats, W. G. T., Moore, J. P. and du Toit, W. J. (2019). Investigating the relationship between cell wall polysaccharide composition and the extractability of grape phenolic compounds into Shiraz wines. Part II: Extractability during fermentation into wines made from grapes of different ripeness levels. *Food Chem.*, 278, 26-35.
- Gómez-Plaza, E., Gil-Muñoz, R., López-Roca, J. M., Martínez-Cutillas, A. and Fernández-Fernández, J. I. (2001). Phenolic compounds and color stability of red wines: Effect of skin maceration time. *Am. J. Enol. Vitic.*, 52(3), 266-270.
- González-Neves, G., Gil, G., Favre, G. and Ferrer, M. (2012). Influence of grape composition and winemaking on the anthocyanin composition of red wines of Tannat. *Int. J. Food Sci. Technol.*, 47, 900-909.
- González-Neves, G., Gil, G. and Barreiro, L. (2008). Influence of grape variety on the extraction of anthocyanins during the fermentation on skins. *Eur. Food. Res. Technol.*, 226, 1349-1355.
- Harbertson, J. F., Mireles, M. S., Harwood, E. D., Weller, K. M. and Ross, C. F. (2009). Chemical and sensory effects of saignée, water addition, and extended maceration on high °Brix must. *Am. J. Enol. Vitic.*, 60 (4), 450-460.



- Harbertson, J. F. and Spayd, S. (2006). Measuring phenolics in the winery. *Am. J. Enol. Vitic.* 57(3), 280-288.
- Hernández-Jiménez, A., Kennedy, J. A., Bautista-Ortín, A. B. and Gómez-Plaza, E. (2012). Effect of ethanol on grape seed proanthocyanidin extraction. *Am. J. Enol. Vitic.*, 63(1), 57-61.
- Ichikawa, M., Ono, K., Matsudo, T. and Okuda, T. (2012). Effect of cap management technique on the concentration of proanthocyanidins in Muscat Bailey A wine. *Food Sci. Technol. Res.*, 18(2), 201-207.
- Iland, P., Ewart, A., Sitters, J., Markides, A. and Bruer, N. (2000). Techniques for chemical analysis and quality monitoring during winemaking (1<sup>st</sup> ed., pp. 1-111) Campbelltown, South Australia: Patrick Iland Wine Promotions.
- Ivanova, V., Vojnoski, B. and Stefova, M. (2012). Effect of winemaking treatment and wine aging on phenolic content in Vranec wines. *J. Food Sci. Technol. Res.*, 49(2), 161-172.
- Kelebek, H., Canbas, A., Selli, S., Saucier, C., Jourdes, M. and Glories, Y. (2006). Influence of different maceration times on the anthocyanin composition of wines made from *Vitis vinifera* L. cvc. Boğazkere and Öküzgözü. *J. Food Eng.*, 77, 1012-1017.
- Koyama, K., Goto-yamamoto, N. and Hashizume, K. (2007). Influence of maceration temperature in red wine vinification on extraction of phenolics from berry skins and seeds of grape (*Vitis vinifera*). *Biosci. Biotechnol. Biochem.*, 71, 958-965.
- Lerno, L., Reichwage, M., Ponangi, R., Hearne, L., Block, D. E. and Oberholster, A. (2015). Effects of cap and overall fermentation temperature on phenolic extraction in Cabernet Sauvignon fermentations. *Am. J. Enol. Vitic.*, 66(4), 444-453.
- Lerno, L. A., Panprivech, S., Ponangi, R., Hearne, L., Blair, T., Oberholster, A. and Block, D. E. (2018). Effect of pump-over conditions on the extraction of phenolic compounds during Cabernet Sauvignon fermentation. *Am. J. Enol. Vitic.*, 3, 295-301.
- Marais, J. (2003). Effect of different wine-making techniques on the composition and quality of Pinotage wine. II. Juice/skin mixing practices. *S. Afri. J. Enol. Vitic.*, 24(2), 76-79.
- Mattivi, F., Vrhovsek, U., Masuero, D. and Trainotti, D. (2009). Differences in the amount and structure of extractable skin and seed tannins amongst red grape varieties. *Aus. J. Grape Wine Res.*, 15, 27–35.
- Monagas, M., Bartolomé, B. and Gómez-Cordovés, C. (2005). Updated knowledge about the presence of phenolic compounds in wine. *Crit. Rev. Food Sci. Nutri.*, 45, 85-118.
- Monagas, M., Gómez, G. and Bartolomé, B. (2005). Evolution of polyphenols in red wines from *Vitis vinifera* L. during aging in the bottle. I. Anthocyanins and pyranoanthocyanins. *Eur. Food. Res. Technol.*, 220, 607-614.
- Mori, K., Saito, H., Goto-Yamamoto, N.G., Kitayama, M., Kobayashi, S., Sugaya, S., Gemma, H. and Hashizume, K. (2005). Effects of abscisic acid treatment and night temperatures on anthocyanin composition in Pinot noir grapes. *Vitis*, 44(4), 161-165.
- Mori, K., Goto-Yamamoto, N., Kitayama, M. and Hashizume, K. (2007). Loss of anthocyanins in red-wine grape under high temperature. *J. Exp. Bot.*, 58(8), 1935-1945.
- Nel, A. P., van Rensburg, P. and Lambrechts, M. G. (2014). The influence of different winemaking techniques on the extraction of grape tannins and anthocyanins. *S. Afri. J. Enol. Vitic.*, 35(2), 304-320.
- Romero-Cascales, I., Fernández-Fernández, J. I., López-Roca, J. M. and Gómez-Plaza, E. (2005). The maceration process during winemaking extraction of anthocyanins from grape skins into wine. *Eur. Food. Res. Technol.*, 221, 163-167.
- Sacchi, K. L., Bisson, L. F. and Adams, D. O. (2005). A review of the effect of winemaking techniques on phenolic extraction in red wines. *Am. J. Enol. Vitic.*, 56(3), 197-206.
- Smith, P. A., Mcrae, J. M. and Bindon, K. A. (2015). Impact of winemaking practices on the concentration and composition of tannins in red wine. *Aus. J. Grape Wine Res.*, 21, 601-614.
- Teixera, A., Eiras-Dias, J., Castellarin, S.D. and Gerós, H. (2013). Berry phenolics of grapevine under challenging environments. *Int. J. Mol. Sci.*, 14, 18711-18739.
- du Toit, W. J. and Visagie, M. (2012). Correlations between South African red grape and wine colour and phenolic composition: Comparing the Glories, Iland and Bovine Serum

Albumin tannin precipitation methods. *S. Afric. J. Enol. Vitic.*, 33(1), 33-41.

Vázquez, E. S., Segade, S. R. and Fernández, I. O. (2010). Effect of the winemaking technique on phenolic composition and chromatic characteristics in young red wines. *Eur. Food. Res. Technol.*, 231, 789-802.

Wold, S., Kettaneh, N., Fridén, H. and Holmberg, A. (1998). Modelling and diagnostics of batch processes and analogous kinetic experiments. *Chemom. Intell. Lab. Syst.*, 44, 331-340.

Zimman, A., Joslin, W., Lyon, M., Meier, J. and Waterhouse, A. (2002). Maceration variables affecting phenolic composition in commercial-scale Cabernet Sauvignon winemaking trials. *Am. J. Enol. Vitic.*, 53(2), 93-98.



# Chapter 3

---

## Supplementary information

**Table S1:** Chemical analysis of wine samples at the end point of alcoholic fermentation before bottling. Samples were analysed with Winescan™ instrument.

Cultivar	Batch	Pressing time	Sample	pH	Volatile Acidity	Total Acid	Malic Acid	Lactic Acid	Glucose	Fructose	Ethanol	Glycerol
CS	2	1	T <sup>a</sup>	3.86 ±0.01	1.42 ±0.09	6.41± 0.12	0.59 ± 0.00	0.30 ± 0.09	1.41 ±0.28	6.52 ± 0.25	14.72±0.08	11.14 ±0.10
CS	2	1	C <sup>b</sup>	3.88 ±0.02	1.4 ± 0.07	6.37 ±0.18	0.6 ± 0.08	0.32 ± 0.13	1.35 ±0.38	5.24 ± 0.42	15.04±0.17	11.03 ±0.21
CS	2	2	T <sup>a</sup>	3.85 ±0.01	1.57 ±0.02	6.51 ±0.01	0.59 ± 0.03	0.21 ± 0.06	1.49 ±0.08	5.34 ± 0.13	14.78±0.00	10.85 ±0.04
CS	2	2	C <sup>b</sup>	3.88 ±0.04	1.51 ±0.16	6.41 ±0.28	0.59 ±0.06	0.18 ± 0.01	1.81 ±0.13	4.99 ± 1.26	14.84±0.31	11.01 ±0.19
CS	2	3	T <sup>a</sup>	3.9 ± 0.00	1.44 ±0.08	6.27 ±0.10	0.55 ±0.04	0.36 ± 0.12	1.01 ±0.06	3.57 ± 0.20	15.00±0.12	10.81 ±0.11
CS	2	3	C <sup>b</sup>	3.84 ±0.00	1.62 ±0.01	6.62 ±0.03	0.71 ±0.01	0.09 ± 0.08	1.81 ±0.09	3.65 ± 0.37	15.11±0.13	10.64 ±0.29
SH	3	1	T <sup>a</sup>	3.61 ±0.02	0.72± 0.04	6.20± 0.27	1.40±0.66	0.19±0.49	2.91±1.52	1.68±0.05	16.47±0.08	11.13±0.03
SH	3	1	C <sup>b</sup>	3.53±0.02	0.61±0.02	6.62±0.03	2.22±0.13	0.05±0.03	1.52±0.14	1.91±0.36	16.43±0.14	10.64±0.13
SH	3	2	T <sup>a</sup>	3.59±0.00	0.70±0.01	6.01±0.03	0.73±0.08	0.7±0.00	1.58±0.37	2.20±1.07	16.23±0.16	11.30±0.20
SH	3	2	C <sup>b</sup>	3.58±0.01	0.68±0.00	6.26±0.04	1.34±0.18	0.38±0.06	1.73±0.05	1.77±0.04	16.43±0.14	11.25±0.11
SH	3	3	T <sup>a</sup>	3.62±0.01	0.76±0.01	5.97±0.04	0.63±0.16	0.75±0.14	1.87±0.17	3.13±0.18	16.36±0.13	11.53±0.12
SH	3	3	C <sup>b</sup>	3.58±0.00	0.74±0.02	6.03±0.06	0.65±0.14	0.69±0.06	1.50±0.01	1.78±0.28	16.19±0.15	11.35±0.15
SH	4	1	T <sup>a</sup>	3.92 ±0.02	0.68±0.05	5.46±0.01	0.63±0.13	1.15±0.05	0.91±0.06	1.61±0.25	15.55±0.40	11.30±0.52
SH	4	1	C <sup>b</sup>	3.91±0.00	0.65±0.01	5.41±0.06	0.70±0.16	1.01±0.23	1.55±0.50	2.14±1.15	15.80±0.19	10.98±0.17
SH	4	2	T <sup>a</sup>	3.89±0.00	0.64±0.01	5.28 ±0.02	0.51±0.10	1.28±0.04	0.87±0.24	2.44±1.44	15.94±0.21	10.69±0.08
SH	4	2	C <sup>b</sup>	3.91±0.02	0.65±0.02	5.22±0.01	0.41±0.01	1.30±0.06	0.98±0.11	2.34±0.18	15.57±0.25	10.67±0.01
SH	4	3	T <sup>a</sup>	3.85±0.04	0.63±0.00	5.31±0.13	0.43±0.11	1.23±0.06	1.19±0.13	2.04±0.86	15.45±0.07	11.00±0.26
SH	4	3	C <sup>b</sup>	3.88±0.03	0.66±0.01	5.40±0.08	0.66±0.01	1.00±0.00	2.01±0.25	2.24±0.11	15.68±0.06	11.42±0.10

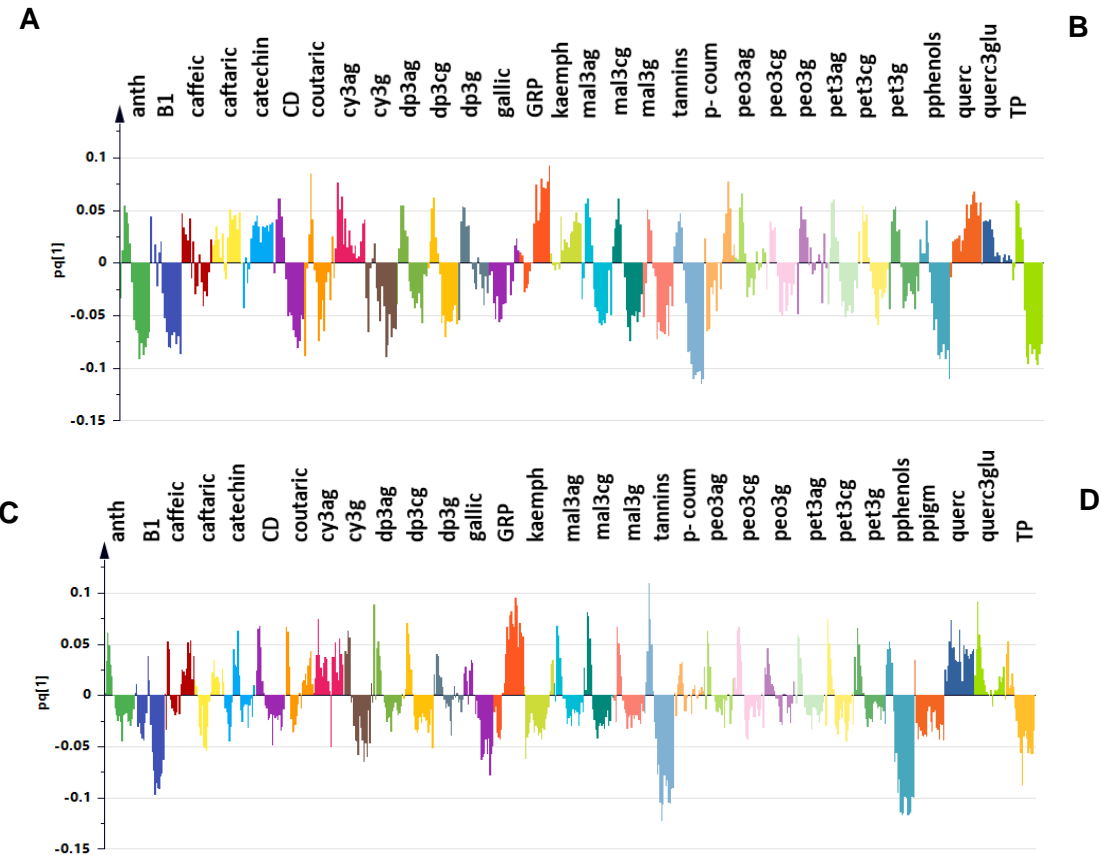
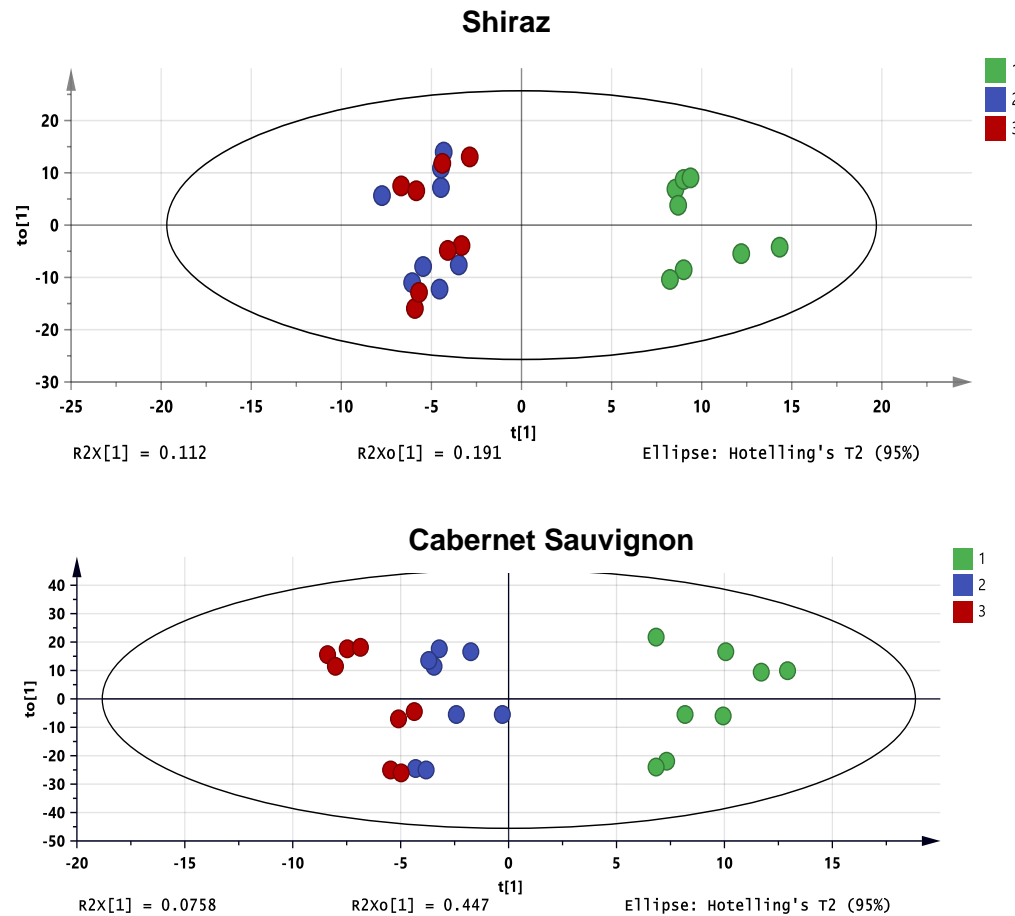
<b>SH</b>	<b>5</b>	<b>1</b>	<b>T<sup>a</sup></b>	<b>3.37±0.06</b>	<b>0.37±0.01</b>	<b>6.51±0.40</b>	<b>1.30±1.09</b>	<b>0.56±0.46</b>	<b>0.72±0.18</b>	<b>1.02±0.30</b>	<b>14.51±0.25</b>	<b>11.17±0.13</b>
SH	5	1	C <sup>b</sup>	3.38±0.05	0.38±0.03	6.45±0.35	1.32±0.84	0.53±0.38	0.76±0.12	1.03±0.04	14.41±0.08	11.02±0.04
SH	5	2	T <sup>a</sup>	3.46±0.01	0.47±0.01	5.85±0.02	0.32±0.04	0.79±0.01	1.07±0.13	1.41±0.10	14.47±0.01	11.32±0.04
SH	5	2	C <sup>b</sup>	3.46±0.02	0.42±0.00	5.91±0.07	0.40±0.01	0.76±0.02	1.20±0.27	1.05±0.06	14.46±0.06	11.52±0.06
SH	5	3	T <sup>a</sup>	3.47±0.01	0.50±0.00	5.84±0.01	0.31±0.01	0.85±0.01	0.98±0.03	1.23±0.01	14.48±0.04	11.34±0.06
SH	5	3	C <sup>b</sup>	3.45±0.01	0.54±0.01	5.89±0.03	0.35±0.02	0.69±0.01	1.36±0.37	1.09±0.09	14.47±0.02	11.46±0.06
CS	6	1	T <sup>a</sup>	3.58±0.01	0.50±0.03	5.84±0.01	0.64±0.07	1.06±0.05	0.70±0.06	1.27±0.14	14.85±0.05	10.61±0.06
CS	6	1	C <sup>b</sup>	3.61±0.01	0.52±0.00	5.82±0.01	0.66±0.02	0.97±0.08	0.46±0.19	1.08±0.15	14.86±0.11	10.66±0.18
CS	6	2	T <sup>a</sup>	3.61±0.01	0.51±0.02	5.72±0.04	0.55±0.02	0.97±0.03	0.60±0.12	0.90±0.05	14.84±0.01	10.67±0.12
CS	6	2	C <sup>b</sup>	3.62±0.01	0.51±0.03	5.67±0.03	0.53±0.04	0.93±0.08	0.47±0.11	1.00±0.01	14.62±0.04	10.63±0.24
CS	6	3	T <sup>a</sup>	3.61±0.01	0.55±0.02	5.69±0.02	0.54±0.02	0.97±0.00	0.47±0.35	1.12±0.19	14.67±0.07	10.49±0.21
CS	6	3	C <sup>b</sup>	3.61±0.00	0.56±0.01	5.71±0.04	0.53±0.06	0.96±0.05	0.34±0.14	1.08±0.04	14.83±0.04	10.66±0.10
SH	7	1	T <sup>a</sup>	3.45±0.00	0.56±0.03	6.41±0.01	2.16±0.05	-0.11±0.07	1.58±0.12	1.90±0.30	16.48±0.16	11.60±0.11
SH	7	1	C <sup>b</sup>	3.43±0.01	0.56±0.03	6.47±0.01	2.11±0.00	-0.165±0.02	1.69±0.06	1.63±0.15	16.48±0.02	11.46±0.06
SH	7	2	T <sup>a</sup>	3.46±0.00	0.55±0.01	6.23±0.01	1.89±0.04	-0.04±0.06	1.71±0.13	1.44±0.04	16.32±0.05	11.38±0.01
SH	7	2	C <sup>b</sup>	3.47±0.02	0.59±0.01	6.20±0.10	1.63±0.10	0.03±0.04	1.68±0.27	1.40±0.02	16.06±0.08	11.47±0.05
SH	7	3	T <sup>a</sup>	3.48±0.05	0.59±0.06	6.16±0.15	1.74±0.30	-0.03±0.13	1.64±0.03	1.68±0.12	16.26±0.01	11.29±0.18
SH	7	3	C <sup>b</sup>	3.46±0.01	0.56±0.02	6.21±0.01	1.74±0.08	-0.02±0.11	1.72±0.13	1.45±0.01	16.31±0.16	11.15±0.13
CS	8	1	T <sup>a</sup>	3.86±0.00	0.53±0.01	5.30±0.01	0.53±0.03	1.33±0.10	-0.41±0.18	1.03±0.06	13.49±0.14	10.16±0.07
CS	8	1	C <sup>b</sup>	3.87±0.01	0.51±0.04	5.34±0.01	0.57±0.08	1.37±0.05	-0.41±0.25	1.03±0.04	13.47±0.02	10.15±0.03
CS	8	2	T <sup>a</sup>	3.84±0.01	0.59±0.07	5.42±0.04	0.55±0.07	1.22±0.23	-0.21±0.85	1.03±0.01	13.24±0.01	10.47±0.04

<b>CS</b>	<b>8</b>	<b>2</b>	<b>C<sup>b</sup></b>	<b>3.85±0.01</b>	<b>0.56±0.01</b>	<b>5.36±0.00</b>	<b>0.52±0.04</b>	<b>1.27±0.01</b>	<b>-0.54±0.04</b>	<b>1.02±0.01</b>	<b>13.12±0.05</b>	<b>10.16±0.03</b>
<b>CS</b>	8	3	T <sup>a</sup>	3.87±0.01	0.62±0.06	5.39±0.06	0.42±0.00	1.37±0.02	-0.82±0.11	1.09±0.02	13.01±0.26	10.39±0.04
<b>CS</b>	8	3	C <sup>b</sup>	3.84±0.01	0.59±0.03	5.36±0.01	0.47±0.01	1.34±0.06	-0.52±0.01	1.01±0.07	13.02±0.04	10.15±0.02
<b>CS</b>	9	1	T <sup>a</sup>	3.77±0.01	0.49±0.01	5.61±0.01	0.73±0.01	0.57±0.01	0.42±0.18	1.21±0.09	14.97±0.04	11.19±0.04
<b>CS</b>	9	1	C <sup>b</sup>	3.74±0.01	0.46±0.01	5.71±0.09	0.80±0.14	0.49±0.08	0.24±0.06	0.96±0.01	14.86±0.29	11.15±0.04
<b>CS</b>	9	2	T <sup>a</sup>	3.78±0.03	0.59±0.09	5.51±0.06	0.59±0.07	0.53±0.06	0.14±0.46	0.96±0.01	14.79±0.25	11.25±0.23
<b>CS</b>	9	2	C <sup>b</sup>	3.82±0.01	0.64±0.07	5.41±0.01	0.45±0.15	0.62±0.14	-0.22±0.23	1.25±0.06	14.98±0.19	11.33±0.15
<b>CS</b>	9	3	T <sup>a</sup>	3.83±0.02	0.73±0.01	5.34±0.02	0.37±0.05	0.66±0.06	-0.30±0.26	1.10±0.01	14.84±0.16	11.08±0.05
<b>CS</b>	9	3	C <sup>b</sup>	3.79±0.01	0.72±0.01	5.43±0.01	0.32±0.09	0.59±0.12	-0.12±0.25	1.02±0.01	14.95±0.06	11.22±0.20

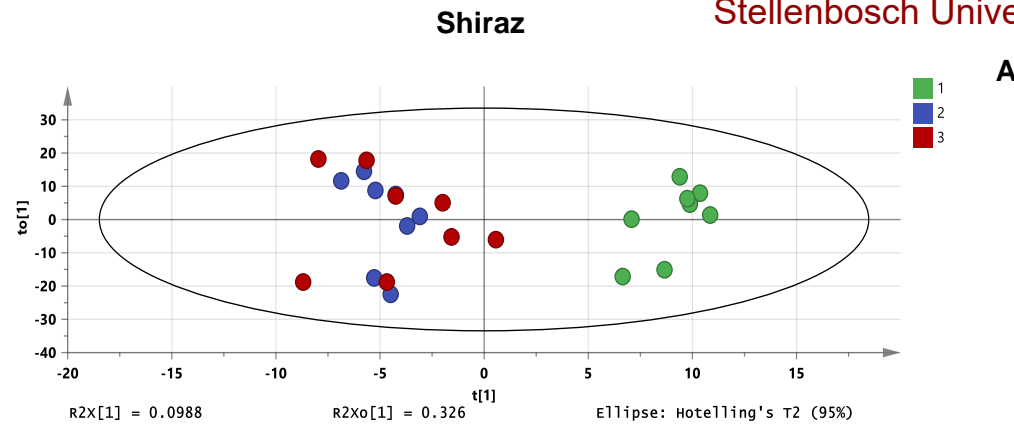
**Table S2:** General analysis of grape batches

Batch	Brix	TA (g/L)	pH
2	23.28±0.18	9.65±0.67	3.63±0.03
3	25.37±0.35	5.70	3.70±0.02
4	26.33±0.26	4.26±0.08	4.10±0.04
5	24.51±0.21	5.12±0.08	3.50±0.03
6	24.92±0.13	4.94±0.10	3.68±0.05
7	26.61±0.15	4.14±0.18	3.47±0.10
8	23.05±0.18	6.05±0.11	3.52±0.05
9	25.30±0.19	4.68±0.06	3.81±0.03

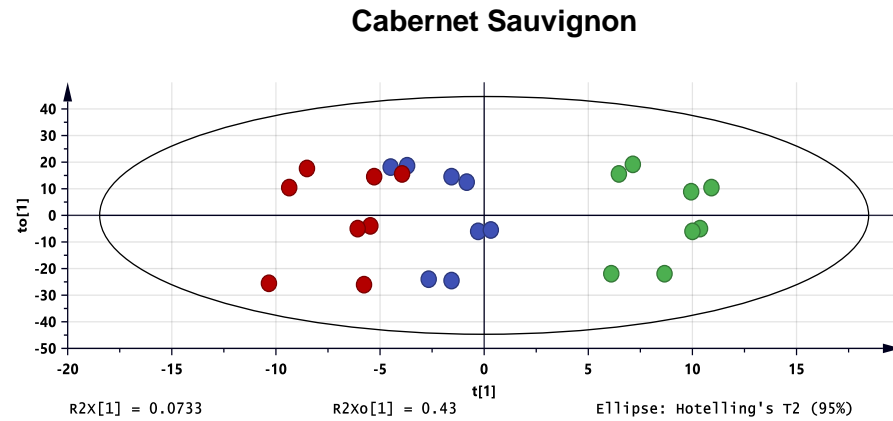
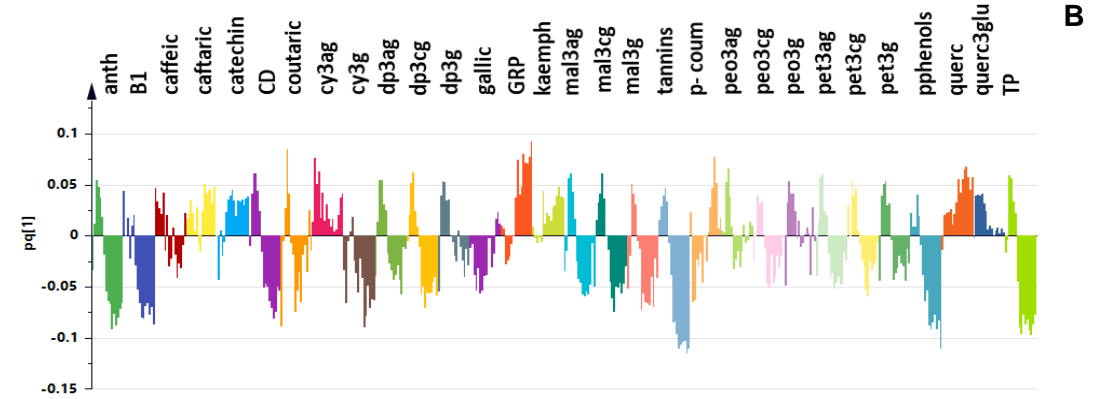
\*CS-Cabernet Sauvignon; SH-Shiraz



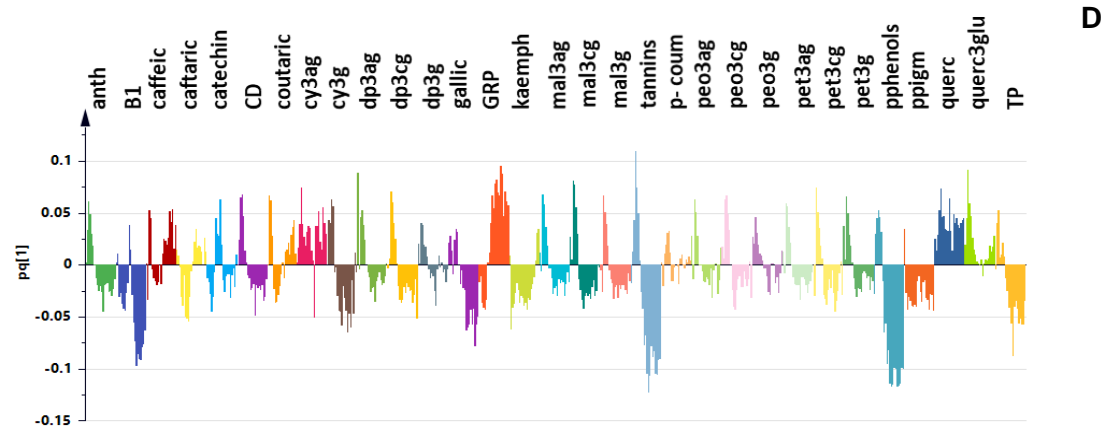
**Figure S1 (A-D):** OPLS-DA score plots discriminating between the three pressing times for Cabernet Sauvignon and Shiraz fermentation samples. **Figure A** and **C** shows the OPLS-DA score plots ( $t_1$  vs  $t_0$ ) for Cabernet Sauvignon and Shiraz fermentation samples pressed at different stages of alcoholic fermentation with enhanced punch down frequency. **Figure B** and **D** shows the corresponding loadings plot ( $p_1$ ) to the OPLS-DA models, coloured according to the measured variables. Each loading observed represents a time point (0-12 days) of the specific phenolic compound/parameter measured. 1-pressing time 1\_ $T^a$ ; 2-pressing time 2\_ $T^a$ ; 3-pressing time 3\_ $T^a$ . Anth: total anthocyanins; B1: dimer B1; caffeic: caffeic acid; cat: catechin; CD: colour density; coutaric: coutaric acid; cy3ag: cyanidin-3-acetylglucoside; cy3g: cyanidin-3-glucoside; dp3ag: delphinidin-3-acetylglucoside; dp3cg: delphinidin-3-cumarylglucoside; dp3g: delphinidin-3-glucoside; gallic: gallic acid; GRP: grape reaction product; kaemph: kaempherol; mal3ag: malvidin-3-acetylglucoside; mal3cg: malvidin-3-cumarylglucoside; mal3g: malvidin-3-glucoside; tannins: total tannin content; *p*-coum: *p*-coumaric acid; peo3ag: peonidin-3-acetylglucoside; peo3cg: peonidin-3-cumarylglucoside; peo3g: peonidin-3-glucoside; pphenols: polymeric phenols; ppigm: polymeric pigments; querc: quercetin; querc3glu: quercetin-3-glucoside; TP: total phenols.



**A**



**C**



**Figure S2 (A-D):** OPLS-DA score plots discriminating between the three pressing times for Cabernet Sauvignon and Shiraz fermentation samples. **Figure A** and **C** shows the OPLS-DA score plots (t1 vs to1) for Cabernet Sauvignon and Shiraz fermentation samples pressed at different stages of alcoholic fermentation with standard punch down frequency. **Figure B** and **D** shows the corresponding loadings plot (p1) to the OPLS-DA models, coloured according to the measured variables. Each loading observed represents a time point (0-12 days) of the specific phenolic compound/parameter measured. 1-pressing time 1\_C<sup>b</sup>; 2-pressing time 2\_C<sup>b</sup>; 3-pressing time 3\_C<sup>b</sup>. Anth: total anthocyanins; B1: dimer B1; caffeic: caffeic acid; cat: catechin; CD: colour density; coutaric: coutaric acid; cy3ag: cyanidin-3-acetylglucoside; cy3g: cyanidin-3-glucoside; dp3ag: delphinidin-3-acetylglucoside; dp3cg: delphinidin-3-cumarylglucoside; dp3g: delphinidin-3-glucoside; gallic: gallic acid; GRP: grape reaction product; kaemph: kaempferol; mal3ag: malvidin-3-acetylglucoside; mal3cg: malvidin-3-cumarylglucoside; mal3g: malvidin-3-glucoside; tannins: total tannin content; *p*-coum: *p*-coumaric acid; peo3ag: peonidin-3-acetylglucoside; peo3cg: peonidin-3-cumarylglucoside; peo3g: peonidin-3-glucoside; pphenols: polymeric phenols; ppigm: polymeric pigments; querc: quercetin; querc3glu: quercetin-3-glucoside; TP: total phenols.



**Table S3:** Analysis of variance (ANOVA) of Shiraz wine samples at the last sampling point (end of alcoholic fermentation). Letters indicate significant difference  $p < 0.05$ .

Compound measured	Pressing time			Punch down level	
	T1	T2	T3	Low	High
anth	611.51±100.78a	700.32±94.21b	681.79±98.58b	659.80±104.a	669.29±104.60a
B1	8.21±4.13c	10.85±3.46b	13.64±3.32a	10.29±4.23a	11.51±4.21a
caffeic	4.56±0.72a	4.72±0.83a	4.81±0.69a	4.88±0.70a	4.51±0.75b
caftaric	28.74±4.65a	29.50±4.56a	26.04±5.98b	28.31±4.53a	27.88±5.91a
cat	13.13±5.98a	11.62±5.96b	11.97±4.76b	11.90±4.98a	12.58±6.09a
CD	20.64±5.31b	24.37±4.82a	23.03±5.31a	21.95±5.37a	23.40±5.19b
coutaric	15.53±3.34ab	16.53±2.60a	15.04±3.20b	15.66±2.42a	15.74±3.65a
cy3ag	3.41±0.46a	3.26±0.41b	3.14±0.28b	3.19±0.41a	3.35±0.38b
cy3g	0.65±0.05b	0.68±0.08ab	0.69±0.78a	0.67±0.09a	0.68±0.08a
dp3ag	5.76±2.47b	6.70±2.17a	6.24±2.04ab	6.25±2.19a	6.22±2.30a
dp3cg	2.43±0.61b	2.93±0.55a	2.86±0.69a	2.79±0.59a	2.69±0.70a
dp3g	13.59±7.01b	16.22±5.96a	14.17±6.36ab	14.23±6.14a	15.16±6.78a
GRP	4.92±0.77a	3.57±1.78b	2.93±0.98c	3.94±1.21a	3.67±1.36a
kaempferol	0.72±0.36a	0.64±0.35b	0.58±0.27b	0.66±0.29a	0.63±0.37a
mal3ag	56.00±12.71b	65.08±14.05a	62.99±11.73a	62.40±12.14a	60.32±14.34a
mal3cg	23.52±4.13a	25.84±3.80a	24.87±5.21a	25.16±3.72a	24.33±5.09a
mal3g	164.58±26.52b	183.09±32.03a	171.95±37.52ab	172.38±26.80a	174.03±38.04a
tannins	1375.42±145.52b	1693.14±191.18a	1707.84±280.91a	1559.79±269.29a	1624.48±251.97a
p-coumaric	0.90±0.49a	0.93±0.40a	0.85±0.36a	0.84±0.45a	0.96±0.37a
peo3ag	8.36±2.65a	8.75±2.78a	7.81±2.43a	8.28±2.23a	8.33±2.97a
peo3cg	6.80±1.86a	7.32±1.62a	6.79±2.22a	7.19±1.85a	6.75±1.94a
peo3g	10.87±3.36a	11.23±4.26a	9.67±3.32b	10.41±3.41a	10.77±3.95a
pet3ag	5.88±2.30b	7.07±2.18a	6.72±2.10a	6.58±2.01a	6.53±2.43a
pet3cg	4.60±1.58a	5.17±1.73a	4.95±1.75a	4.94±1.55a	4.87±1.81a
pet3g	19.06±6.97b	22.99±6.00a	20.45±6.72b	20.69±6.08a	20.98±7.29a

<b>polymeric phenols</b>	865.87±63.15b	1031±129.52a	1050.95±159.49a	965.97±141.94a	999.48±154.20a
<b>querc</b>	7.60±3.40a	6.71±3.08b	5.33±2.32c	6.58±3.05a	6.51±3.11a
<b>querc3glu</b>	68.09±28.66b	72.24±29.82a	72.68±29.94a	70.08±28.49a	71.92±29.94a
<b>TP</b>	51.68±6.24b	59.30±5.71a	57.07±7.60a	55.16±6.81a	56.88±7.66a

\*T<sup>a</sup> enhanced punch down frequency; C<sup>b</sup> standard punch down frequency

\*CS- Cabernet Sauvignon; SH- Shiraz

**Table S4:** Analysis of variance (ANOVA) of Cabernet Sauvignon wine samples at the last sampling point (end of alcoholic fermentation). Letters indicate significant difference  $p < 0.05$ .

Compound measured	Pressing time			Punch down level	
	T1	T2	T3	Low	High
anth	599.53±180.93b	678.77±160.52a	668.28±158.41a	645.97±173.07a	651.75±164.70a
caftaric	16.70±7.05a	17.71±7.40a	14.55±7.15b	17.45±7.69a	15.20±6.58b
cat	11.81±5.42b	12.22±5.56b	15.08±7.71a	12.23±6.12a	13.85±6.60b
CD	21.34±7.75b	25.15±7.25a	24.33±6.56a	23.37±7.68a	23.83±6.93a
coutaric	7.62±4.91a	7.92±5.31a	6.81±5.10a	7.87±5.30a	7.0±4.81a
cy3ag	4.15±0.43ab	4.25±0.21a	4.07±0.45b	4.17±0.39a	4.15±0.36a
cy3g	0.54±0.09b	0.63±0.06a	0.64±0.06a	0.60±0.08a	0.61±0.09a
dp3ag	5.80±2.83b	6.67±3.75a	6.78±3.62a	6.41±3.55a	6.43±3.27a
dp3cg	1.72±0.80b	2.11±1.17a	2.11±1.10a	2.05±1.06a	1.93±1.02a
gallic	7.21±4.30b	8.26±4.61b	12.03±4.77a	8.64±4.69a	9.70±5.20a
kaempferol	0.58±0.26a	0.53±0.36ab	0.47±0.34b	0.53±0.32a	0.52±0.32a
mal3ag	45.87±22.78b	54.69±23.58a	55.00±24.96a	51.90±24.97a	51.80±22.84a
mal3cg	15.56±8.00b	18.33±9.64a	18.79±9.54a	17.28±9.24a	17.83±8.97a
mal3g	142.33±39.50b	162.05±47.47a	167.02±55.27a	156.94±51.02a	157.33±45.97a
tannins	1573.47±401.86b	2023.35±272.15a	2067.73±254.14a	1832.42±408.92b	1943.73±354.99a
p-coumaric	1.40±0.53b	1.62±0.79a	1.53±0.74ab	1.50±0.74a	1.52±0.65a
peo3ag	5.30±2.13b	6.22±3.26a	5.96±3.07a	6.02±3.01a	5.63±2.68a
peo3g	9.32±2.83a	9.88±2.66a	10.28±3.57a	9.77±3.16a	9.88±2.91a
pet3cg	5.66±2.71b	6.69±3.46a	6.62±3.63a	6.57±3.22a	6.11±3.36a
pet3cg	2.93±1.27b	3.83±1.80a	3.70±1.83a	3.53±1.71a	3.47±1.67a
pet3g	15.50±8.67b	19.07±9.03a	18.57±10.37ab	17.54±9.82a	17.80±8.93a

<b>polymeric pigments</b>	7.24±2.78b	6.30±3.64b	9.70±4.28a	7.23±3.77a	8.27±3.92b
<b>quercetin</b>	7.20±3.15a	6.42±2.82b	5.45±2.80c	6.17±3.12a	6.54±2.82b
<b>quercetin3glu</b>	36.54±12.22b	40.53±12.74a	39.32±12.42ab	37.36±12.21a	40.23±12.51b
<b>TP</b>	53.03±12.30b	60.32±9.47a	62.16±10.65a	57.21±11.83a	59.80±10.96b

\*T<sup>a</sup> enhanced punch down frequency; C<sup>b</sup> standard punch down frequency

\*CS- Cabernet Sauvignon; SH- Shiraz

# Chapter 4

---

## **General conclusions and recommendations**

## 4.1 GENERAL CONCLUSIONS

Phenolic compounds are important bio-substances in red wine, influencing organoleptic properties as well as aging potential. Nevertheless, since the mention of the “French paradox” by researchers, i.e. the positive influence of regular red wine consumption on human health, consumers have increased their investments in the product, with quality becoming of the outmost importance. Moreover, red wine quality has been defined by colour and mouthfeel attributes, with anthocyanins and condensed tannins playing key roles. Considering this, research has been conducted to possibly optimize phenolic content during the fermentation process. The importance of managing the maceration length was highlighted as the most important variable to possibly modify phenolic content of the final wine (Casassa and Harbertson, 2014). In addition, to management of skin contact time, skin/juice mixing techniques was also reported as an influential variable, however limited research was in this case conducted (Ichikawa *et al.*, 2012). Regardless of winemaking practices or techniques used in industry to modify phenolic content, the influence of grape variety itself also needs to be taken into consideration, since phenolic extractability, composition and content appears to be variety dependent. Taking all of this into account, a suitable, rapid, accurate, simple and cost-effective methodology is a necessity to monitor phenolic extraction during red wine fermentations. Several attempts have been investigated to quantify phenolic levels of fermenting samples through spectroscopy calibrations to evaluate phenolic extraction during the fermentation process. Limitations in phenolic analyses and data processing have probably been contributing to limited phenolic analyses being performed on a frequent scale during red wine fermentations. However, an on-line monitoring system predicting phenolic content as well as alert possible deviations during the maceration process would be an ideal approach for future red wine fermentations. This will hopefully help to grow the wine industry as well as to improve overall red wine quality and competitiveness with international producers

The main aim of this study was therefor to frequently analyse a wide array of phenolic compounds in Shiraz and Cabernet Sauvignon fermentations, exposed to different pressing and punch down regime and process the resulting data. First off, all, the influence of grape variety on phenolic extraction was addressed by randomly selecting eight batches with a varying range of phenolic content from the Western Cape wine region. Phenolic differences were observed evaluating the effect of skin presence during different stages of maceration between Cabernet Sauvignon and Shiraz fermentations. Results showed more clear trends for Cabernet Sauvignon fermentations when exposed to longer maceration times than Shiraz. Overall, fermentations pressed at pressing time 2 and 3 showed trends of higher phenolic content compared to vinifications pressed at 1/3<sup>rd</sup> of fermentation. Taking everything into account, phenolic extraction was a diffusion process driven by the maceration length, however final content was batch dependent.

Next, evaluating the results of the punch down regimes, cultivar differences were observed indicating possible batch dependent results. Increased punch downs showed trends of high phenolic content in comparison with standard punch downs only associated with high phenolic acid content. Moreover, punch down seems to have a positive effect in the tannin fraction with variation in the results observed that might be attributed to cultivar differences.

However, further investigation is needed to determine if punch down is a suitable cap management approach to enhance phenolic extraction, since results seem variety dependent.

Furthermore, BSPC showed to be a suitable, rapid and accurate approach to monitor phenolic kinetics during alcoholic fermentation. Clear trends of phenolic extraction were observed in the corresponding loadings plot for both cultivars i.e. anthocyanin extraction was first associated with wines pressed at 1/3<sup>rd</sup> of fermentation, but as fermentation progressed higher anthocyanin levels were associated with later pressing times. However, as fermentation nearly finished anthocyanin levels slightly decreased probably due to refixation on the skins, precipitation or participation in other reactions. Again, as seen in the corresponding loadings plots, phenolic behaviour was driven by the presence/absence of grape skins. Optimum contact between the skins and the juice was crucial for the diffusion of phenolics from the skins to the grape juice. Management of the maceration length was key in modifying phenolic content.

The current study proved the suitability of PLS calibrations to quantify phenolic content of a large number of fermenting samples obtained during maceration as well as to estimate accurate levels in line with current research. Moreover, management of the maceration length proved to be a powerful tool to modify phenolic content regardless of phenolic variability between grape batches. The presence of skins during different stages of maceration influenced phenolic extraction. However, phenolic differences were observed between Cabernet Sauvignon and Shiraz indicating Cabernet Sauvignon as being more suitable for longer maceration conditions. In addition, the approach of batch statistical process control proved to be a suitable monitoring tool for red wine fermentations with the easy detection of possible deviations during the process. Furthermore, BSPC may be used in future red wine productions to possibly modify phenolic content and improve overall red wine quality.

## 4.2 FUTURE RECOMMENDATIONS

- i. The lack of research investigating the effect of skin/must mixing techniques on phenolic content in commercial and large-scale fermentations of South African red cultivars needs to be addressed with further studies to confirm the influence of enhanced contact between the solids and the liquids as well as determine the best suitable method to improve overall red wine quality. In addition, to determine if the results are grape or batch dependent for each method.
- ii. Evaluate the vintage effect in our current study, since phenolic content can vary from the same vineyard every year due to environmental and cultural practices influencing phenolic accumulation and content.
- iii. Monitor red wine fermentations on-line with batch statistical process control as a monitoring tool to modify phenolic content during the process. Data should be logged frequently to monitor the process.
- iv. Investigate phenolic evolution during aging to determine if the phenolic content modified during maceration is still significantly different after aging.



- v. Evaluate the influence of presence/absence of skins during different stages of maceration on the sensorial properties of red wine.